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Fluorescein diacetate vital staining allows earlier diagnosis of rifampicin-resistant tuberculosis

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SUMMARY

SETTING: Damien Foundation Project, Bangladesh.

OBJECTIVE: To evaluate sputum smear fluorescein diacetate (FDA) vital staining to predict culture-defined failure and rifampicin (RMP) resistance.

DESIGN: A retrospective, operational study.

RESULTS: A total of 1633 episodes of auramine smear-defined late conversion and failure could be evaluated (respectively 640 and 584 on first treatment and 185 and 224 on retreatment). Negative FDA was 95% predictive of negative culture in patients on first treatment, while its positive predictive value was around 95% during retreatment. The predictive value of a positive (not scanty) result for RMP resistance or environmental non-tuberculous mycobacteria (NTM) was at least 90%, except in late converters on first-line treatment; a negative result was over 95% exclusive of the same except in retreatment failures. FDA correctly identified 88–98% of all RMP resistance.

CONCLUSIONS: FDA staining increased the proportion of tuberculosis patients put on second-line treatment without receiving the standard first-line retreatment regimen. In our setting, with excellent microscopy, late case presentation and low resistance prevalence, it proved indispensable for efficient culture and referrals of early suspects for rapid drug susceptibility testing (DST). In other settings with low prevalence of NTM and difficult access to accurate and rapid DST, FDA-positive failures might even be considered for immediate start of second-line treatment.

KEY WORDS: microscopy; multidrug resistance; viability; culture; drug susceptibility testing

IN CONTEXTS with effective anti-tuberculosis treatment and low rates of primary multidrug-resistant tuberculosis (MDR-TB), excellent microscopy for acid-fast bacilli (AFB) may lead to frequent over-diagnosis of first treatment failure, due to confusion with late excretion of dead bacilli.[1,2] National control programmes (NTPs) often attempt to screen late AFB-smear converters for MDR-TB, but this is highly inefficient.[3] Culture and drug susceptibility testing (DST) will generally fail, while line-probe assay (LPA) DST may yield inconclusive results due to the presence of degraded DNA.

We previously reported the high predictive value of vital staining with fluorescein-diacetate (FDA) for the diagnosis of true (culture-proven) treatment failure, showing over 95% correlation with growth in culture. Based on this study conducted in a reference laboratory, we recommended FDA for MDR-TB screening.[4] Since 2008, the FDA technique has been used in conjunction with DST on slides (slide DST)[4] by four regional laboratories for earlier switch to the highly efficient Bangladesh gatifloxacin second-line regimen.[5] This operational study aimed to retrospectively evaluate the usefulness of FDA screening by referral indication in terms of its predictive values with mycobacterial culture and DST as references, and its overall contribution to MDR-TB management.

The Damien Foundation (DF) Bangladesh Project implements TB control on behalf of the NTP in a population of about 25 million people who often present with advanced but drug-susceptible disease.[6,7] Regimens with rifampicin (RMP) throughout are used for new (Category I) and retreatment cases (Category II). Treatment is directly observed, using all possible providers.[8] In accordance with NTP guidelines, treatment failure is declared from 5 months of treatment onwards, even for a single AFB. The same cut-off is used for the definition of smear-positive relapse (at least one AFB in one sputum smear at any time after cure or treatment completion).

METHODS

Patients and specimens

Initially, all types of MDR-TB suspects were eligible for FDA smear: Category I and II failures and relapses

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as well as late converters (smear-positive after respectively 3 and 4 months of an extended intensive phase), with any number of AFB on Ziehl-Neelsen (ZN) or auramine smear. Samples were generally examined fresh, referring the patient to a regional FDA and slide DST laboratory, where auramine microscopy was repeated in conjunction with FDA staining. Another specimen was collected in cetylpyridinium chloride preservative for culture on Löwenstein-Jensen (LJ) medium at the project reference laboratory. LJ DST was performed at the Antwerp supranational reference laboratory (SRL) on systematically referred isolates.

FDA procedure

The following modifications to our previous technique were introduced:

- staining unfixed smears for 30 min with FDA working solution, 3 min destaining with 0.5% acid alcohol and 10 min with phenol 5% for sterilisation;[4]
- quenching with 0.5% aqueous potassium permanganate for 1 min after destaining;
- reading one smear length at 200× magnification on a light-emitting diode (LED) fluorescence microscope (rather than the 1000× required by mercury vapour systems), using FLUOLED® Easy module (Fraen Corporation Srl, Settimo Milanese, Italy) with 450 nm LED and filters for auramine on Olympus CX21 or CX31 microscopes (Olympus Corporation, Tokyo, Japan);
- preventing precipitation and evaporation from less concentrated stocks (500 μl FDA stock aliquots at 0.5% weight/volume in acetone, instead of 100 μl at 5%);
- adding a positive control smear from a newly diagnosed smear-positive case.

Reference laboratory tests

Decontamination, primary culture and DST on LJ were performed using standard methods, as previously described.[6] Our slide DST technique was slightly modified from the earlier description: [4]

- sputum was homogenised with *N*-acetylcysteine/citrate for even smearing;
- selective antibiotics replaced acid decontamination;[9]
- Middlebrook 7H9 with 10% goat's serum replaced Sula medium;
- a para-nitrobenzoic acid 500 μg/ml bottle was added for the identification of NTM.[10]

NTM identification relied on p-nitrobenzoic acid growth and microcolony morphology on slide DST, in addition to 16S r-RNA analysis on LJ isolates referred to the SRL.

Ethics considerations

Because of its retrospective nature, the study was not submitted to an ethical committee.

RESULTS

Table 1 shows an overview of all 4512 FDA tests performed by the four laboratories during these 3 years by indication. Relapses after Category I are underrepresented, as this indication was soon dropped considering the high number and the low yield of MDR-TB. FDA smears were performed for 1477 Category I and 292 Category II late converters, and 748 and 317 failures. The percentage of negative results varied from almost 80% among late Category I converters to 21% for Category II relapses. Scanty positive results (converted to 1–9 per 100 high-power fields)[11] made up 41% of all FDA-positive smears from late converters, and less in the other groups.

Table 2 shows the results of FDA staining compared with liquid and/or solid medium culture results (as far as available; contamination excluded) for the 2834 late converters and smear-defined failures only. One result was counted per patient/indication, keeping the most positive result in case of discordance. The specificity and negative predictive value (NPV) of FDA were high among Category I late converters and failures (respectively $n = 640$, specificity 91% and NPV 95% and $n = 584$, specificity 95% and 97% NPV). Sensitivity and positive predictive value (PPV) were higher for Category II patients (93% and 94% among 185 late converters, 94% and 97% among 224 failures). Sensitivity was only around 80–85% for Category I samples, but the PPV was lowest (69% for late converters and 83% for failures); on Category II, specificity among failures was 91%, while the NPV remained moderate, at 83%, with slightly better values for late converters (93% specificity, 91% NPV).

Table 3 shows RMP-resistant (Rr) TB and NTM detected by indication and FDA result, and the PPV for both together, for culture-positive specimens

Table 1 Numbers of FDA vital smears performed, by indication and result

	FDA smear results				
Indication	Positive* *n*	Scanty* *n*	Negative *n*	Total *n*	Negative %
Category I late conversion	177	123	1177	1477	79.7
Category I failure	129	57	562	748	75.1
Category II late conversion	128	49	115	292	39.4
Category II failure	180	52	85	317	26.8
Category I relapse	126	39	82	247	33.2
Category II relapse	333	144	125	602	20.8
Other†	275	121	433	829	52.2
Total	1348	585	2579	4512	57.2

*Scanty, positive: refers to the International Union Against Tuberculosis and Lung Disease/World Health Organization quantification scale for acid-fast smears.
†Includes new, return after default, second-line treatment monitoring and unspecified.
FDA = fluorescein diacetate.

Table 2 FDA vital staining operating characteristics in acid-fast smear late converters and treatment failures (samples with positive or negative culture result available only; one result per patient and indication)

	FDA-negative		FDA-positive or scanty positive					
Indication	Culture-positive *n*	Culture-negative *n*	Culture-positive *n*	Culture-negative *n*	Sensitivity % (95%CI)	Specificity % (95%CI)	PPV % (95%CI)	NPV % (95%CI)
Category I late conversion ($n = 640$)	26	468	100	46	79 (71–86)	91 (88–93)	69 (60–76)	95 (92–96)
Category I failure ($n = 584$)	16	441	105	22	87 (79–92)	95 (93–97)	83 (75–86)	97 (94–98)
Category II late conversion ($n = 185$)	7	74	98	6	93 (86–97)	93 (84–97)	94 (87–98)	91 (82–96)
Category II failure ($n = 224$)	10	49	160	5	94 (89–97)	91 (79–97)	97 (93–99)	83 (71–91)

FDA = fluorescein diacetate.

only. About 92–95% of the isolates from Category II samples with any number of viable AFB and from at least 1+ positive Category I failures were Rr or NTM. For Category I FDA-positive late converters and scanty-positive failures, this PPV was around 80%, yet with scanty positive FDA smears from late Category I converters the PPV reached only 44%. Not shown in the table are (ZN-positive) FDA-negatives showing growth on culture: 58% of 26 Category I and 57% of seven Category II FDA-negative but culture-positive late converter specimens yielded Rr or NTM isolates, compared with 88% of 16 Category I and 90% of 10 Category II failures.

Table 4 shows the diagnostic accuracy for viable AFB, Rr and NTM (all samples). Few Rr or NTM were isolated from FDA-negative samples, yielding a 95–97% correct prediction of clinically unimportant AFB positivity, except in the group of Category II failures (85%). NTM represented about half of the missed late converters on both regimens; however, missed failures were usually Rr. Scanty positive FDA smears were poorly predictive of Rr for Category I late converters (16% Rr, no NTMs identified in this category) or failures (29% Rr, 45% when including NTM). Scanty positive FDA smears were more predictive during Category II, particularly considering Rr TB and NTM together (75–87%). A positive FDA smear indicated Rr TB in 67% of late Category I converters (no NTM in this group), increasing to 82–88% in the other groups (or 90–93% adding the NTM). Overall accuracy of the FDA smears in including or excluding Rr TB or NTM was around 90% in all groups except Category I late converters (86%, 95% confidence interval [CI] 83–89). Of all Rr, 90%/88% were FDA-positive or scanty among Category I late converters/failures respectively and 98%/96% among Category II late converters/failures. Overall, this was 93% (95%CI 90–95). These proportions were slightly lower (91%, 95%CI 88–93%) when NTM was included.

Table 5 shows all registrations for MDR-TB treatment during 2005–2010. The percentage of all patients diagnosed with Rr effectively put on treatment rose from 55% in 2005 to nearly 90% in 2010. Particularly in 2009 (34%) and 2010 (46%), a larger

Table 3 FDA vital staining PPVs for RMP-resistant TB and environmental mycobacteria in acid-fast smear late converters and treatment failures, limited to samples growing mycobacteria in solid and/or liquid medium

Indication, FDA result	Susceptible TB *n*	RMP-resistant TB *n*	NTM *n*	PPV for RMP-resistant TB or NTM % (95%CI)
Category I late conversion				
Scanty ($n = 18$)	10	8	0	44 (22–69)
Positive ($n = 82$)	18	64	0	78 (67–86)
Both ($n = 100$)	28	72	0	72 (62–80)
Category I failure				
Scanty ($n = 21$)	4	11	6	81 (57–94)
Positive ($n = 84$)	4	73	7	95 (88–98)
Both ($n = 105$)	8	84	13	92 (85–96)
Category II late conversion				
Scanty ($n = 19$)	1	11	7	95 (72–100)
Positive ($n = 79$)	5	70	4	94 (85–98)
Both ($n = 98$)	6	81	11	94 (87–97)
Category II failure				
Scanty ($n = 34$)	2	24	8	94 (79–99)
Positive ($n = 126$)	9	107	10	93 (86–96)
Both ($n = 160$)	11	131	18	93 (88–96)

FDA = fluorescein diacetate; PPV = positive predictive value; RMP = rifampicin; TB = tuberculosis; NTM = non-tuberculous mycobacteria; CI = confidence interval.

Table 4 FDA vital staining accuracy in excluding or predicting RMP-resistant TB and environmental mycobacteria in acid-fast smear late converters and treatment failures

Parameter	Category I late conversions *n* or % (95%CI)	Category I failures *n* or % (95%CI)	Category II late conversions *n* or % (95%CI)	Category II failures *n* or % (95%CI)	Total *n* or % (95%CI)
FDA-negative					
Total	494	457	81	59	1091
RMP-resistant TB	8	11	2	6	27
NTM	7	3	2	3	15
True negative or non RMP-resistant TB	97 (95–98)	97 (95–98)	95 (87–98)	85 (73–92)	96 (95–97)
FDA scanty					
Total	50	38	24	37	149
RMP-resistant TB	8	11	11	24	54
NTM	0	6	7	8	21
Correct RMP-resistant TB	16 (8–30)	29 (16–46)	46 (26–67)	65 (47–79)	36 (29–45)
Correct RMP-resistant TB or NTM	16 (8–30)	45 (29–62)	75 (53–89)	87 (70–95)	50 (42–59)
FDA-positive					
Total	96	89	80	128	393
RMP-resistant TB	64	73	70	107	314
NTM	0	7	4	10	21
Correct RMP-resistant TB	67 (56–76)	82 (72–89)	88 (78–94)	84 (76–89)	80 (76–84)
Correct RMP-resistant TB or NTM	67 (56–76)	90 (81–95)	93 (84–97)	91 (85–95)	85 (81–89)
Any FDA result					
Total	640	584	185	224	1633
Accuracy RMP-resistant TB or NTM	86 (83–89)	92 (90–94)	91 (86–95)	89 (84–93)	89 (88–91)
RMP-resistant TB flagged	90 (81–95)	88 (80–94)	98 (91–100)	96 (90–98)	93 (90–95)
RMP-resistant TB or NTM flagged	83 (73–90)	87 (79–93)	96 (89–99)	94 (89–97)	91 (88–93)

FDA = fluorescein diacetate; RMP = rifampicin; TB = tuberculosis; CI = confidence interval; NTM = non-tuberculous mycobacteria.

proportion of these were diagnosed early, based on FDA and slide DST, before Category II treatment was given. A slightly higher proportion could not be confirmed by conventional DST as Rr in 2008 (13%) and 2009 (10%), but in 2010 this proportion had fallen to the previous level (7%).

DISCUSSION

We have previously reported excellent results with FDA vital staining on freshly examined sputum samples, reaching 92% PPV and 97% NPV for growth in culture.[4] These results were obtained in a reference laboratory on fresh specimens from Category I failures. We recommended the technique, followed by rapid slide DST, for early switch to second-line treatment and limiting referrals for DST to patients with FDA-positive sputum samples. There have been virtually no other reports on the use of FDA for TB, and to the best of our knowledge only our own project in Bangladesh uses this screening and early switch strategy. We report here our experience with the decentralised, routine use of FDA performed on all MDR-TB suspects identified over a 3-year period. Since then, the technique has improved: LED fluorescence microscopy was found to work better than conventional fluorescence microscopy, as it permits scanning at 200× using the standard auramine excitation lamp and emission filter.

The proportion of patients with confirmed Rr who switched to second-line treatment increased from 60% to 83% of diagnosed cases. Moreover, the proportion of patients with confirmed Rr who were identified and who switched to second-line treatment before receiving Category II treatment tripled. Initially, a slightly higher number of cases were falsely diagnosed; however, this was soon reduced to the previous low levels, considered acceptable as the standard Bangladesh regimen is less toxic and cheaper. Moreover, our unpublished results show that the Bangladesh regimen produces equally good outcomes for non-confirmed MDR-TB (RMP- and/or isoniazid-susceptible TB) compared to the reported results for MDR-TB.[5]

Table 5 Trends of enrolment for MDR-TB treatment, Damien Foundation Bangladesh, 2005–2010

	2005 *n* (%)	2006 *n* (%)	2007 *n* (%)	2008 *n* (%)	2009 *n* (%)	2010 *n* (%)
RMP-resistant cases diagnosed	101	106	167	127	129	121
RMP-resistant enrolled (% of diagnosed)	66 (65)	69 (65)	106 (64)	96 (76)	111 (86)	108 (89)
RMP-resistant enrolled before Category II (% of enrolled)	3 (5)	2 (3)	15 (14)	16 (17)	38 (34)	50 (46)
Erroneously considered RMP-resistant (% of enrolled)	5 (8)	4 (6)	6 (6)	12 (13)	11 (10)	8 (7)

MDR-TB = multidrug-resistant tuberculosis; RMP = rifampicin.

We have limited this report to FDA performance on AFB-positive sputum samples from late converters and failures during standard first-line treatment, although other retreatment groups, such as relapses, were also initially targeted. Among these, FDA has the highest PPV, but fairly low NPV for viability (data not shown). Moreover, while Category I relapse rarely represents MDR-TB, Category II relapses in our population comprised equal proportions of pan-susceptible TB, MDR-TB and NTM. FDA was thus soon stopped for relapsed patients; Category I relapses were started on the Category II regimen with simultaneous slow DST; however, rapid-slide DST was performed for Category II relapses.

Our analysis shows that FDA screening may also be less useful in Category II failures with an unsatisfactory NPV for growth (83%, 95%CI 71–91) and 90% of false-negative FDA representing Rr or NTM. Rapid DST and identification without FDA screening could thus be recommended for Category II failures, which have been reported to represent over 80% of MDR-TB cases from many populations.[12]

FDA screening proved most useful in Category I late converters and failures. An NPV of at least 95% would allow us to avoid more than 60% of all DST referrals in our population. The small percentage with viable bacilli missed could be re-tested at a later occasion if still positive. Close to half of FDA-negative culture-positive late converters on Category I grew RMP-susceptible TB. A scanty FDA result may be ignored in case of these late converters awaiting further evolution, as most would not grow or yield susceptible bacilli, with only 16% predictive value for Rr.

The FDA PPV was high among Category I failures and Category II late conversions, a clearly positive (not scanty) result reaching 90% for Rr or NTM. In settings without NTM or with difficult DST access, a clearly positive FDA result could thus be a sufficient indication to enrol a patient on cost-efficient MDR-TB treatment. Given the logistical complications of DST, such as false-negative cultures, DST errors and mortality due to difficult and lengthy referrals, settings with access to DST may also benefit from FDA. A strategy for switching to second-line treatment based on positive FDA smears in failures and late retreatment converters would be even more justified in settings with higher MDR-TB prevalence among new cases than in our population, where it was only 0.5%. Differentiation between Rr and NTM would then be the main reason for further testing in settings where NTM are not very rare in these groups of MDR-TB suspects. This is not reliable using microscopy,[13] and a false diagnosis of NTM particularly might have serious consequences.

The Bangladesh NTP guidelines made better use of our FDA results impossible. The vast majority of FDA-negative Category I failures may not have needed the first-line retreatment regimen to which they had to be automatically switched, even when only one AFB was found. The current World Health Organization (WHO) guidelines for TB control programmes recommend starting empirical second-line treatment for first-line treatment failures while awaiting the results of conventional slow DST, or when DST is not possible.[14] In our case, this would have meant that over half of all second-line treatment regimens were given to cured patients.

Our study had some limitations. First, due to lack of data, we were unable to calculate the predictive value for relapse, which might have been indicated for late converters (failures had already been switched to the next higher treatment regimen based on AFB smear). It would seem likely that late conversion FDA-positive patients with RMP-susceptible TB are at increased risk for relapse with standard treatment. We have recently shown that extension of the intensive phase of Category I treatment does not prevent failures, but significantly reduces relapse among smear-positives at 2 months of a regimen with RMP throughout. However, the predictive value for relapse of conventional AFB smears was only about 2%.[15] Half of the 3-month FDA-positive Category I cases in this study had RMP-susceptible TB, and it is possible that only these really benefited from intensive phase extension.

Second, the reference for growth on culture and Rr included slide DST, which is not a WHO-recommended method. However, our previous study yielded very similar predictive values for growth on LJ culture only, and most samples reported here underwent slide DST as well as LJ DST at the SRL, with 90–95% agreement for Rr in the various laboratories. Considering any Rr as the true result might thus have led to slight over-diagnosis, meaning that the PPV of FDA for Rr might have been slightly over- and the NPV slightly under-estimated.

The FDA technique is simple, but has some requirements, albeit far fewer than culture. With LED fluorescence microscopes, the instrument and power supply no longer pose serious problems. However, FDA powder must be stored frozen at −20°C, as are stock solutions (or kept at 4°C for a few months), limiting its decentralised use in most settings to the intermediate level laboratory. Moreover, sputum needs to be fresh or kept refrigerated.

CONCLUSION

In contrast with conventional acid-fast smears, positive FDA results late during treatment were a strong predictor of failure to convert to negative culture and of RMP resistance. Decentralised use of the technique tripled the proportion of TB patients started early on second-line treatment. FDA vital staining can thus greatly improve the efficiency of MDR-TB screening and diagnosis among smear-defined late converters

and first-time failures in settings with a low prevalence of RMP resistance, late case presentation and/or highly sensitive acid-fast microscopy. With already high pre-test MDR-TB probability, a positive FDA test could even be considered as sufficient evidence to start second-line treatment, particularly with a well-tolerated regimen and low prevalence of NTM.

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RÉSUMÉ

CONTEXTE : Projet de la Fondation Damien au Bangladesh.
OBJECTIF : Evaluer la coloration vitale au diacétate de fluorescéine (FDA) pour les frottis de crachats en matière de prédiction de l'échec défini par la culture et de prédiction de la résistance à la rifampicine (RMP).
SCHÉMA : Etude opérationnelle rétrospective.
RÉSULTATS : On a pu évaluer 1633 épisodes de négativation tardive et d'échec définis par la coloration du frottis à l'auramine (respectivement 640 et 584 cas de premier traitement et 185 et 224 cas de retraitement). Une FDA négative a une valeur prédictive de 95% pour une culture négative chez les patients sous premier traitement, alors que sa valeur prédictive positive se situe autour de 95% en cas de retraitement. La valeur prédictive d'un résultat positif (à l'exclusion des très faiblement positifs) pour une résistance à la RMP ou la présence de mycobactéries environnementales (NTM) est d'au moins 90%, sauf en cas de négativation tardive au cours du premier traitement ; un résultat négatif permet d'exclure plus de 95% des mêmes données, sauf en cas d'échec de retraitement. La FDA a identifié correctement entre 88% et 98% de l'ensemble des cas de résistance à la RMP.
CONCLUSION : La coloration FDA augmente la proportion de patients TB mis sous traitement de deuxième ligne, sans qu'un régime de retraitement standard de première ligne ait été mis en route. Dans notre contexte, où l'examen microscopique est excellent, la présentation des cas tardive et la prévalence des résistances faible, elle s'est avérée indispensable pour la référence rapide à une culture efficiente et à la DST des cas précocement suspects. Dans d'autres contextes où la prévalence des NTM est faible et l'accès difficile à des tests précis et rapides de sensibilité, on pourrait même prendre en considération la mise en route immédiate d'un traitement de deuxième ligne dans les échecs positifs à la FDA.

RESUMEN

MARCO DE REFERENCIA: El proyecto de la Damien Foundation en Bangladesh.
OBJETIVO: Evaluar la utilidad de la baciloscopia de esputo con tinción por diacetato de fluoresceína para evaluar la vitalidad celular, en la predicción del fracaso terapéutico definido por el cultivo y la resistencia a rifampicina (RMP).
MÉTODO: Fue este un estudio operativo retrospectivo.
RESULTADOS: Se pudieron evaluar 1633 episodios de conversión tardía y fracaso terapéutico definidos mediante la tinción con auramina (640 conversiones tardías y 584 fracasos en tratamientos iniciales y 185 conversiones tardías y 224 fracasos en pacientes en retratamiento). Un resultado negativo de la coloración con FDA pronosticó 95% de cultivos negativos en pacientes en tratamiento inicial y el valor pronóstico de un resultado positivo fue de 95% durante el retratamiento. El valor pronóstico de un resultado positivo (con excepción de las baciloscopias con bacilos escasos) para resistencia a RMP o presencia de micobacterias atípicas (NTM) fue como mínimo de 90%, con la excepción de las conversiones tardías de un tratamiento inicial. Un resultado negativo fue excluyente de las mismas en más del 95%, excepto en los fracasos del retratamiento. La tinción con diacetato de fluoresceína detectó en forma correcta de 88% a 98% de todas las resistencias a RMP.
CONCLUSIONES: La FDA aumentó la proporción de pacientes en quienes se administró un tratamiento de segunda línea sin haber recibido la pauta de retratamiento corriente de primera línea. En el entorno del estudio, con un servicio excelente de microscopia, una presentación tardía de los casos y una baja prevalencia de resistencia, esta prueba demostró ser indispensable para una eficacia máxima de los cultivos y remisiones rápidas con el fin de realizar pruebas de sensibilidad a los medicamentos en los casos con presunción temprana de resistencia. En otros medios, con baja prevalencia de NTM y dificultad de acceso a antibiogramas precisos y rápidos, los casos de fracaso con un resultado positivo a la FDA se podría incluso considerar el comienzo inmediato de un tratamiento de segunda línea.