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Saliva-based linezolid monitoring on a mobile UV spectrophotometer

Hannah Yejin Kim^{1,2,3*}, Evelien Ruiter^{1,4†}, Erwin M. Jongedijk⁵, Hemanth Kumar AK⁶, Ben J. Marais^{3,7}, Bhavani PK⁶, Shailendra Sawleshwarkar^{3,8}, Daan J. Touw⁵ and Jan-Willem Alffenaar^{1,2,3}

¹Sydney Pharmacy School, Faculty of Medicine and Health, The University of Sydney, Camperdown, NSW, Australia; ²Westmead Hospital, Westmead, NSW, Australia; ³Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, Camperdown, NSW, Australia; ⁴School of Pharmacy, Utrecht University, Utrecht, The Netherlands; ⁵University of Groningen, University Medical Center Groningen, Department of Clinical Pharmacy and Pharmacology, Groningen, The Netherlands; ⁶National Institute for Research in Tuberculosis, Chennai, India; ⁷Department of Infectious Diseases and Microbiology, The Children's Hospital at Westmead, Westmead, Australia; ⁸Westmead Clinical School, Faculty of Medicine and Health, The University of Sydney, Westmead, NSW, Australia

*Corresponding author. E-mail: hannah.kim@sydney.edu.au

†Contributed equally.

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Background: In TB, therapeutic drug monitoring (TDM) is recommended for linezolid; however, implementation is challenging in endemic settings. Non-invasive saliva sampling using a mobile assay would increase the feasibility of TDM.

Objectives: To validate a linezolid saliva assay using a mobile UV spectrophotometer.

Methods: The saliva assay was developed using NanoPhotometer NP80® and linezolid concentrations were quantified using second-order derivative spectroscopy. Sample preparation involved liquid–liquid extraction of saliva, using saturated sodium chloride and ethyl acetate at 1:1:3 (v/v/v). The assay was validated for accuracy, precision, selectivity, specificity, carry-over, matrix effect, stability and filters. Acceptance criteria were bias and coefficient of variation (CV) <15% for quality control (QC) samples and <20% for the lower limit of quantification (LLOQ).

Results: Linezolid concentrations correlated with the amplitude between 250 and 270 nm on the second-order derivative spectra. The linezolid calibration curve was linear over the range of 3.0 to 25 mg/L ($R^2 = 0.99$) and the LLOQ was 3.0 mg/L. Accuracy and precision were demonstrated with bias of –7.5% to 2.7% and CV ≤5.6%. The assay met the criteria for selectivity, matrix effect, carry-over, stability (tested up to 3 days) and use of filters (0.22 µm Millex®-GV and Millex®-GP). Specificity was tested with potential co-medications. Interferences from pyrazinamide, levofloxacin, moxifloxacin, rifampicin, abacavir, acetaminophen and trimethoprim were noted; however, with minimal clinical implications on linezolid dosing.

Conclusions: We validated a UV spectrophotometric assay using non-invasive saliva sampling for linezolid. The next step is to demonstrate clinical feasibility and value to facilitate programmatic implementation of TDM.

Introduction

Global commitments to end TB have only seen a 9% decline in TB incidence rate in the last 5 years, far below the 2020 milestone target of 20%. The WHO End TB Strategy and UN Sustainable Development Goals have set targets of a 50% decline in TB incidence rate by 2025 and ending the global TB epidemic by 2030.¹ Worldwide, approximately half a million cases of rifampicin-resistant TB (RR-TB) were reported in 2019, of which 78% were MDR-TB.¹ Management of RR-TB and MDR-TB is challenging and treatment is long and cumbersome, taking 9–20 months.² Clearly, there is a need to improve treatment requiring not only more

active drugs but also better tolerated drugs.^{3–5} Linezolid together with fluoroquinolones (levofloxacin and moxifloxacin) and bedaquiline form WHO Group A category drugs in the MDR-TB regimen.² Group A drugs are considered highly effective and are recommended for inclusion in MDR-TB regimens.² Optimal exposure to linezolid ensures maximal efficacy and suppression of acquired drug resistance, and the established pharmacokinetic/pharmacodynamic (PK/PD) target is $AUC_{0-24}/MIC >100-119$.^{6,7} However, toxicity limits its use and mitochondrial dysfunction, such as peripheral neuropathy, optic neuropathy and bone marrow suppression, is associated with linezolid exposure.⁸ Clinical studies have

endeavoured to find the optimal linezolid dosing strategies to maximize efficacy and minimize toxicity.^{9–11}

In TB, therapeutic drug monitoring (TDM) has been recommended for linezolid due to its narrow therapeutic index and drug exposure-related outcomes.^{2,6,7,12} Despite the evidence and recommendations, implementing linezolid TDM in TB endemic settings has challenges.^{13,14} Many WHO-listed high-TB burden countries lack funding for laboratory facilities and skilled staff for performing HPLC-UV or LC-MS/MS assays. Additionally, sample logistics do not facilitate a short turnaround time. TDM can only be operated effectively if there are programmatic strategies and a framework for TDM in such low-resource settings.¹⁵

To increase the feasibility of TDM during TB treatment, alternative sampling matrices have been explored, such as dried blood spots, saliva and urine.^{16–19} These methods are non-invasive and allow ambulatory sampling. Due to the small sample volume, dried blood spot analysis requires sensitive LC-MS/MS equipment, which is rarely available. Saliva is an attractive matrix for clinical application as linezolid has a substantial penetration into saliva with a saliva/plasma ratio of 0.76–0.97 observed for linezolid saliva concentrations of up to about 15 mg/L.^{16,20} Clinical studies have reported a good correlation between plasma and saliva concentrations based on full concentration–time curves using LC-MS/MS to measure linezolid in saliva.^{16,20} Although HPLC-UV is easier to perform than LC-MS/MS, it still requires skilled laboratory scientists, which does not facilitate access to TDM in local healthcare settings. More promising for programmatic implementation of TDM is the use of a mobile UV spectrophotometer.¹⁸ Use of equipment requires a drug to be present in saliva in the mg/L range and have a UV absorption spectrum. This is true for linezolid and potentially would increase the local accessibility to drug assays and further increase the feasibility of TDM in diverse settings.

Our study aimed to develop and validate an assay to quantify linezolid in saliva using a mobile UV spectrophotometer in order to enable linezolid TDM in low-resource TB endemic settings.

Materials and methods

Materials

Acetaminophen, clofazimine, D-cycloserine, ethyl acetate, isoniazid, levofloxacin, linezolid, meropenem, moxifloxacin, *p*-aminosalicylic acid, pyrazinamide, rifampicin, sodium chloride and trimethoprim were purchased from Sigma-Aldrich, Australia. Abacavir, azithromycin, emtricitabine, ethambutol, ethionamide, fluconazole, lamivudine, nevirapine, sulfamethoxazole and zidovudine were purchased from Sapphire Bioscience, Australia. All chemicals were of $\geq 98\%$ purity. Ultrapure water (resistivity at 25°C = 18.2 M Ω ·cm) was obtained using a Milli-Q® Integral Water Purification System (Merck Millipore, Australia).

The samples were measured on a UV spectrophotometer, NanoPhotometer NP80® (Implen, Germany), using disposable cuvettes (70–850 μ L, 8.5 mm, Brand® UV cuvettes). The UV spectrophotometer was set on wavescan mode with a detection range of 200–900 nm, pathlength of 10 mm, baseline correction and smoothing filter switched off.

Method development

Lambert–Beer's law states that light absorbance is directly proportional to the concentration of the sample.²¹ For a drug following this principle, zero-order absorbance spectra would correlate with the drug concentration. However, the response measured at the wavelength of the maximum

absorbance (λ_{max}) can be impacted by interferences from co-medications and endogenous compounds. For linezolid, derivative spectroscopy was applied as a strategy to increase the selectivity and specificity of the detection as previously described.¹⁸ The amplitude of a second-order derivative of the spectrum is expected to correlate with drug concentration and was calculated for linezolid by polynomial fitting using the Savitsky–Golay method.²² The polynomial order and wavelength interval were optimized for measured amplitude and minimal signal noise.

Sample preparation

Saliva (for method development and validation) was collected from six healthy individuals who chewed on a purpose-made cotton roll (Salivette®, Sarstedt, Australia) for 2 min. The Salivette® tube was centrifuged at 4000 rpm for 2 min to obtain saliva. Sample clean-up was optimized by testing different extraction conditions of the previous method²³ to reduce interference from saliva and to further increase selectivity and specificity of the Savitsky–Golay method. Liquid–liquid extraction involved mixing 300 μ L of saliva with 300 μ L of saturated sodium chloride solution and 900 μ L of ethyl acetate (1:1:3 v/v/v). The mixture was vortexed for 1 min and centrifuged at 13 000 rpm for 5 min; 630 μ L of the supernatant was taken and evaporated with nitrogen at 30°C (TurboVap® Classic LV from Biotage, John Morris Scientific Pty Ltd). After evaporation, 140 μ L of ultrapure water was added and the sample was vortexed for 30 s. The samples (100 μ L) were measured with disposable cuvettes on the UV spectrophotometer.

Assay validation

Validation of the assay was performed in accordance with the current guidelines of the EMA and the FDA guidelines for bioanalytical method validation.^{24,25}

Two separate stock solutions of linezolid 1000 mg/L were prepared in pooled saliva from six individuals. Each solution was used to prepare calibration standards (3.0, 4.0, 6.0, 8.0, 12, 16, 20 and 25 mg/L) and quality control (QC) samples at the lower limit of quantification (LLOQ), low, medium and high (3.0, 6.0, 12 and 20 mg/L). All samples were prepared using the optimized liquid–liquid extraction as described above.

Accuracy (bias%) and precision (coefficient of variation; CV%) were determined by analysing the QC samples in five replicates on three different days. Carry-over was assessed by measuring LLOQ samples directly after high QC samples. Selectivity was tested on saliva from six individuals either drug-free or spiked at linezolid LLOQ. Matrix effect was assessed using the drug-free saliva from six individuals, first extracted then spiked at low and high QC. Specificity was determined by interferences from potential co-medications at linezolid LLOQ and high QC using pooled saliva. Second-line TB drugs (clofazimine, D-cycloserine, ethambutol, ethionamide, isoniazid, levofloxacin, meropenem, moxifloxacin, *p*-aminosalicylic acid, pyrazinamide and rifampicin), antiretroviral drugs (abacavir, emtricitabine, lamivudine, nevirapine and zidovudine) and other drugs that are either common or likely in TB/HIV patients (acetaminophen, amoxicillin, azithromycin, fluconazole, metformin, sulfamethoxazole and trimethoprim) were selected. Tested concentrations were based on previously reported C_{max} in saliva or plasma if data on saliva penetration were not available. Filter validation was carried out using 0.22 μ M Millex®-GV (polyvinylidene difluoride) and Millex®-GP (polyethersulfone) (Merck Millipore, Australia) at LLOQ, low, medium and high QC. For stability, linezolid low and high QC samples made in saliva were stored in a refrigerator (2–8°C), at room temperature (20–25°C) and in a stove (37°C) and analysed on Day 2 and Day 3 of the validation. Carry-over, selectivity, matrix effect and stability were tested in triplicates. Accuracy and precision for the three calibration curves, filter validation and specificity from interfering drugs were tested in five replicates.

Acceptance criteria for all tested parameters was bias and CV <15% for low, medium and high QC and <20% for LLOQ.

Results

Linezolid saliva samples spiked at clinically relevant concentrations of 3.0 to 25 mg/L showed absorbance spectra with baseline shifts and increasing magnitudes of absorbance at λ_{max} of 250 nm (Figure 1, left-hand panel). These magnitude differences on zero-order spectra showed a lack of correlation with linezolid concentrations. However, when the second-order derivative spectra were plotted, the amplitude between 250 and 270 nm correlated with linezolid concentration (Figure 1, right-hand panel). Calculation of the second-order derivative by the Savitsky-Golay method is affected by changes in the wavelength interval and order of the polynomial fit. For linezolid, a fourth-order polynomial fitted with a filter strength of 16 was the optimal setting to maximize the measured amplitude with minimal signal noise. At the higher concentrations of 20 and 25 mg/L, peak splitting was observed; however, the amplitude of the second-order derivative resulted in linearity in calibration curves ($R^2=0.99$) from linezolid 3.0 to 25 mg/L (Figure 2). The LLOQ was 3.0 mg/L.

A substantial inter-subject variability was observed in drug-free saliva. As high as 50% variability in absorption at λ_{max} 280 nm was noted due to endogenous compounds in saliva. Sample clean-up and recovery was optimized. Recovery was lower in acidic extraction (1.0 M hydrochloric acid) compared with neutral and alkaline extraction (2.0 M sodium hydroxide) (Figure S1, available as [Supplementary data](#) at JAC Online). The final ratio between saliva, saturated sodium chloride and ethyl acetate was determined to be 1:1:3 (v/v/v). Evaporation of ethyl acetate using nitrogen gas resulted in an improved signal-to-noise ratio and calibration curve compared with using an evaporator at 70°C (Figure S2).

Within-day and between-day accuracy and precision tested for linezolid 3.0, 6.0, 12 and 20 mg/L showed acceptable CV and bias of <15% (<20% for LLOQ) (Table 1). Bias ranged from –7.5% to

2.7%, within-day CV was $\leq 1.7\%$ and between-day and overall CV was $\leq 5.6\%$ for all tested QC samples. Selectivity of the assay was demonstrated by drug-free saliva from six healthy individuals showing responses below linezolid LLOQ and by spiked individual samples showing an acceptable CV of 14% at 3.0 mg/L (LLOQ). Matrix effect from individual saliva was low yielding a CV of 6.7% and 2.5% at 6.0 and 20 mg/L, respectively. Carry-over effect was minimal with bias and CV $\leq 1\%$ at 3.0 mg/L measured directly after 20 mg/L.

Filter validation using 0.22 μM Millex®-GV and Millex®-GP resulted in accurate and precise quantification of linezolid QC samples at 3.0, 6.0, 12 and 20 mg/L (Table 1). The use of Millex®-GV showed low CV of $\leq 1.2\%$ and bias of –8.3% to 6.3%. Similarly, Millex®-GP resulted in CV $\leq 1.0\%$ and bias ranging from –12.9% to 3.9%.

Figure 2. Calibration curve ($n=3$) of linezolid.

Figure 1. Spectra of linezolid 3.0, 4.0, 6.0, 8.0, 12, 16, 20 and 25 mg/L in saliva. Zero-order spectra (left-hand panel) and second-order derivative spectra (right-hand panel) with Savitsky-Golay filter (filter strength=16 and polynomial order=4). Amplitude differences in the second-order derivative spectra correlate with linezolid concentrations.

Table 1. Validation results of linezolid saliva assay on NanoPhotometer NP80® including filter validation (using 0.22 µM Millex®-GV and Millex®-GP)

Criterion	Linezolid							
	LLOQ, 3.0 mg/L		low, 6.0 mg/L		medium, 12 mg/L		high, 20 mg/L	
Accuracy (bias%)	2.7		-4.7		-7.5		-3.9	
Within-day precision (CV%)	1.1		1.2		1.3		1.7	
Between-day precision (CV%)	5.1		4.0		4.2		5.6	
Overall precision (CV%)	4.5		3.6		3.8		5.1	
Selectivity at LLOQ (CV%)	14.3		-		-		-	
Matrix effect (CV%)	-		6.7		-		2.5	
Carry-over at LLOQ (CV%, bias%)	0.9, 1.0		-		-		-	
	CV%	Bias%	CV%	Bias%	CV%	Bias%	CV%	Bias%
Millex ® -GV	1.2	6.3	0.5	-4.2	0.9	-8.3	1.2	-3.2
Millex ® -GP	1.0	3.9	0.5	-6.0	0.7	-12.9	0.6	-6.8

Accuracy and precision for the three calibration curves: in five replicates.

Filters: in five replicates.

Selectivity, matrix effect and carry-over: in triplicates.

Interference from potential co-mediations was tested (Table 2). Lacking saliva $C_{\max}$ of pyrazinamide, we tested the $C_{\max}$ from plasma assuming a 1:1 ratio as the worst-case scenario. Pyrazinamide 40 mg/L interfered significantly with linezolid 3.0 and 20 mg/L (bias of 775.1% and 34.3%, respectively). A more than proportional reduction in bias was observed when pyrazinamide 20 mg/L was tested. The subsequent bias at linezolid 3.0 mg/L was 224.2% and at linezolid 20 mg/L was -17.8%. No significant interference was observed when mimicking pyrazinamide trough concentrations of 5.0 and 10 mg/L (CV $\leq$ 2.3%, bias -6.7 to 1.5%). Similarly, higher concentrations of levofloxacin 15 mg/L and moxifloxacin 5.0 mg/L interfered with linezolid 3.0 mg/L with a bias of 36.9% and 21.8%, respectively. However, lower concentrations of levofloxacin 7.5 mg/L and moxifloxacin 3.0 mg/L reduced bias to 16.8% and 12.4%, respectively. Both levofloxacin and moxifloxacin at all tested concentrations showed minimal interference at linezolid 20 mg/L (bias $\leq$ 13.9%). Rifampicin resulted in a slightly high bias of 24.9% and 36.9% for linezolid 3.0 and 20 mg/L, respectively. Of the tested antiretroviral drugs, abacavir resulted in a slightly high bias of 32.8% at linezolid 3.0 mg/L, but minimal interference with a bias of 2.1% when tested with linezolid 20 mg/L. Of other common drugs, acetaminophen 12.5 mg/L interfered significantly with a bias of 198.2% at linezolid 3.0 mg/L and 28.2% at linezolid 20 mg/L. Acetaminophen at a trough concentration of 5.0 mg/L still showed a significant interference, although with a reduced bias of 93.8% at linezolid 3.0 mg/L. Trimethoprim 5.0 mg/L also interfered with linezolid 3.0 mg/L with a borderline bias of -20.9%. All other drugs showed minimal interferences, demonstrated by acceptable CV and bias $<$ 15% at linezolid 20 mg/L and $<$ 20% at 3.0 mg/L.

The stability of linezolid saliva samples was tested under different storage conditions (Table 3). Linezolid 6.0 and 20 mg/L stored in a refrigerator (2–8°C), at room temperature (20–25°C) and in a stove (37°C) were stable on Day 2 and Day 3 (CV $\leq$ 2.3%, bias -10.5% to -3.3%).

Discussion

We successfully validated an assay to measure linezolid in saliva samples on a mobile UV spectrophotometer. To our knowledge, this is the first saliva assay for linezolid developed using a mobile UV spectrophotometer¹⁸ that could facilitate TDM of linezolid in local healthcare settings. The assay met criteria in terms of accuracy, precision, selectivity, matrix effect, carry-over and specificity.

Linezolid saliva samples also remained stable for the first 3 days tested, even at high temperature of 36.7°C, which is relevant for endemic settings with extremes of weather conditions and a lack of refrigeration/freezing facilities. The validated filters will also provide safe handling strategies for culture/smear-positive patient samples.²⁶ For example, saliva samples can be filtered at a patient's bedside at home, reducing the need for transporting infectious samples to the laboratory.

Interferences from some of the tested TB and HIV drugs were noted; however, limited clinical impact can be expected. The observed interference from pyrazinamide is from peak pyrazinamide on the trough linezolid concentration, hence the interference would be lower if we were to simulate the drugs being given at the same time. Pyrazinamide trough concentrations of 5.0 and 10 mg/L showed no marked interferences with linezolid. Furthermore, pyrazinamide is a WHO Group C drug and is recommended only when Group A and B drugs cannot complete the regimen,² thus implying a lower likelihood for the concurrent use of linezolid and pyrazinamide. It is interesting to note that interference from pyrazinamide at 42 mg/L was also observed with levofloxacin when measured in saliva.¹⁸ However, the extent of interference was lower compared with linezolid as $\lambda_{\max}$ for pyrazinamide is closer to that of linezolid. Although we estimated pyrazinamide saliva concentration to be comparable to concentrations in plasma based on low protein binding it would be of value to actually measure the saliva/plasma ratio to inform interference testing for future assays.

Co-administration with levofloxacin and moxifloxacin showed interference at linezolid LLOQ. Linezolid is likely to be used with

Table 2. Interference test for potential co-medications (tested in five replicates)

		Linezolid LLOQ (3.0 mg/L)		Linezolid high (20 mg/L)	
	Tested concentration (mg/L)	CV%	bias%	CV%	bias%
TB drugs					
clofazimine	0.5	0.5	12.5	0.8	1.8
D-cycloserine	25	0.8	10.2	0.4	−4.1
ethambutol	5.0	1.0	13.4	1.2	−0.5
ethionamide	2.5	1.2	10.4	3.2	8.7
isoniazid	15	1.0	5.6	1.7	−0.3
levofloxacin	7.5	2.0	16.8	2.6	13.8
	10	0.7	33.1	3.2	13.9
	15	0.7	36.9	1.1	6.2
meropenem	5.0	1.0	9.7	1.8	−1.0
	30	0.8	13.0	1.6	−3.7
moxifloxacin	3.0	0.9	12.4	2.2	7.4
	5.0	1.2	21.8	2.2	11.5
p-aminosalicylic acid	20	0.4	8.2	1.9	−2.3
pyrazinamide	5.0	1.9	1.5	1.5	−5.6
	10	0.8	−6.7	2.3	−5.5
	20	0.4	224.2	2.8	−17.8
	40	2.5	775.1	3.8	34.3
rifampicin	12.5	2.0	24.9	5.6	36.9
Antiretroviral drugs					
abacavir	2.5	0.8	32.8	2.3	2.1
emtricitabine	1.0	1.3	9.1	1.6	3.0
lamivudine	1.0	0.8	6.7	0.6	1.4
nevirapine	5.0	2.1	−3.4	3.9	3.2
zidovudine	1.0	0.5	4.5	1.3	−0.9
Other drugs					
acetaminophen	5.0	1.9	93.8	1.1	30.3
	12.5	0.9	198.2	4.2	28.2
amoxicillin	10	1.1	14.3	3.8	8.1
azithromycin	0.5	0.7	14.5	4.1	1.6
fluconazole	10	0.8	9.6	3.9	−0.8
metformin	2.0	0.6	14.1	2.1	4.7
sulfamethoxazole	10	1.6	11.3	6.8	10.2
trimethoprim	5.0	1.7	−20.9	3.3	3.9

Note: Guideline criteria for acceptable bias and CV% is <20% for LLOQ and <15% for high QC.

these fluoroquinolones and bedaquiline, as they are WHO Group A drugs, recommended for MDR-TB or RR-TB.² However, the level of interference at the linezolid trough concentration implies low clinical significance (3.0 mg/L reported as 3.7–4.1 mg/L) and only when these drugs are at their peak concentrations at the same time as linezolid is present at the trough concentration. This is highly unlikely when drugs are taken at the same time, especially when applying direct observed therapy as recommended by the WHO.² The small differences in the absolute value are unlikely to result in a clinical decision for dose change, especially as linezolid C_{max} is higher, around 15–27 mg/L.⁶

Concurrent use of rifampicin with linezolid is unlikely as the current recommendation for isoniazid-monoresistant (rifampicin-susceptible) TB is to replace isoniazid with fluoroquinolones.²

Therefore, the observed interference from rifampicin was considered to have a low clinical relevance.

Interference from acetaminophen, a common analgesic, was noted at both low and high linezolid concentrations. However, acetaminophen has a short half-life of 1.5 to 2.5 h²⁷ and morning saliva sampling for linezolid could account for minimal detection of acetaminophen in saliva.

Despite the observed inter-patient variability in drug-free saliva, the use of derivative spectroscopy and optimized sample preparation were able to account for this effect. A limitation of our assay is the use of cuvettes as opposed to an easier nano-volume drop tray.¹⁸ However, cuvettes allowed increased detection sensitivity, which was required since a liquid-liquid extraction was applied.

Table 3. Stability of linezolid low (6.0 mg/L) and high (20 mg/L) QC samples in saliva in different storage conditions [in a refrigerator (2–8°C), at room temperature (20–25°C) and in a stove (37°C)] (tested in triplicates)

	Day 2				Day 3			
	6.0 mg/L		20 mg/L		6.0 mg/L		20 mg/L	
	CV%	bias%	CV%	bias%	CV%	bias%	CV%	bias%
Refrigerator (2–8°C)	1.3	–10.0	1.1	–9.0	0.7	–6.7	0.5	–3.3
Room temperature (20–25°C)	0.5	–8.6	2.3	–8.2	0.7	–7.5	0.7	–3.9
Stove (37°C)	1.0	–10.5	1.8	–8.4	0.1	–7.1	1.1	–3.9

The limit of detection of our linezolid assay is 3.0 mg/L, which is higher than the limit of detection reported for LC-MS/MS (e.g. 0.05 mg/L).¹⁶ However, the established PK/PD target for linezolid is an AUC_{0–24}/MIC ratio >119⁶ and limited sampling strategies could be developed for saliva as has been done in the past for plasma samples²⁸ to use minimal sampling timepoints for AUC prediction. Hence, the need for trough sampling and detection below 3.0 mg/L may not be necessary with such sampling strategies. In those situations where it would be required, an additional dried blood spot sample could be collected and sent to a reference laboratory.¹⁹

Importantly, the mobile UV spectrophotometer could be used in remote and regional settings and is a great benefit that could enable TDM of linezolid in remote settings. The mobile assay can help to establish a framework for TDM, not only for linezolid but for other drugs as well.^{29–31} Often, in TB endemic settings, TDM is not an option, as access to LC-MS/MS in clinical practice is limited, even in large cities. In addition to non-invasive saliva sampling, a highly affordable, mobile UV spectrophotometer will allow drug measurement at local healthcare facilities without the need to transport samples to central laboratories. There will be a significant reduction in the long turnaround time and clinicians can make prompt dose adjustment based on drug exposure.

The next step would be to clinically validate the linezolid saliva assay on the UV spectrophotometer in MDR-TB patients. Previous studies have demonstrated a good correlation between saliva and plasma linezolid samples measured by LC-MS/MS.^{16,20} Patients' saliva samples measured by UV spectrophotometry would need to be compared with the saliva and plasma samples measured by HPLC-UV or LC-MS/MS. Dose recommendations made based on the results from the different matrixes and assays should be compared.

Dosing strategies for linezolid are under continuous revision and studies are inconclusive about the preferred regimen.^{6,7} This underlines the added value of linezolid TDM and ideally accompanied by drug susceptibility to assess the relative drug exposure based on the AUC/MIC ratio.^{32,33}

In conclusion, we validated the accuracy of saliva testing using a mobile UV spectrophotometer to assess linezolid levels. The availability of screening assays using non-invasive saliva samples should increase the accessibility and feasibility of programmatic implementation of TDM, in settings where advanced quantitative assays, such as LC-MS/MS, are not available.^{15,34} Implementation studies are required to demonstrate its clinical feasibility and value to guide optimal linezolid dosing of MDR-TB patients.

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Transparency declarations

None to declare.

Supplementary data

Figures S1 and S2 are available as [Supplementary data](#) at JAC Online.

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