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Whole genome sequencing for drug-resistant tuberculosis management in South Africa: What gaps would this address and what are the challenges to implementation?

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ABSTRACT

Global control of tuberculosis (TB) has been seriously impacted by the emergence and transmission of its drug-resistant forms. Delayed detection and incomplete characterisation of drug-resistant tuberculosis (DR-TB) contributes to morbidity and mortality, and to ongoing transmission of drug-resistant strains. Current culture-based and molecular diagnostic tools for TB present numerous disadvantages that could potentially lead to misdiagnosis, inappropriate treatment initiation and the amplification of drug resistance. The detection of drug-resistant tuberculosis (DR-TB) in South Africa relies on molecular diagnostic assays such as the Xpert MTB/RIF and line probe assays (MTBDR*plus* and MTBDR*sl*). However, these molecular assays are limited to detecting resistance to only a few first-line and second-line drugs. It is for this reason that next-generation sequencing (NGS) and bioinformatics pipelines have been developed for rapid detection of *M. tuberculosis* drug resistance, with the added advantage that sequence data could also have public health applications through understanding transmission patterns. This review highlights some of the challenges that are currently hampering the diagnosis and control of DR-TB in a high burden setting of the KwaZulu-Natal (KZN) province in South Africa. Shortfalls of current diagnostic techniques for DR-TB are discussed in detail and we also propose how these might be overcome with an accurate and rapid NGS system.

1. Introduction

Despite a major reduction in tuberculosis (TB) cases and deaths in the past decade, the disease continues to be a major global public health problem [1]. In 2017, 10 million people were estimated to have developed TB worldwide. Human immunodeficiency virus (HIV) co-infection, the emergence and transmission of drug-resistant tuberculosis (DR-TB) and delayed diagnosis and treatment have exacerbated this problem. According to the World Health Organization (WHO), an estimated 558 000 new cases of rifampicin-resistant tuberculosis (RR-TB) were reported globally in 2017, with 82% of these cases resistant to both rifampicin (RIF) and isoniazid (INH) or multidrug-resistant tuberculosis (MDR-TB). The latest data further reveals that 3.5% of new and 18% of previously treated TB cases had MDR-TB or RR-TB, while 8.5% of MDR-TB cases had extensively drug-resistant tuberculosis (XDR-TB), which is resistant to RIF, INH, any fluoroquinolone and at least one of the second-line injectable drugs (SLIDs) [1].

Drugs currently used to treat DR-TB are expensive, toxic, require a long duration and have very low successful treatment outcomes [1]. At least 82% of global drug-susceptible TB cases are treated successfully, while success rates have been reported to be at 55% for MDR-TB [1]. DR-TB can be diagnosed through a number of tests, such as culture-based drug susceptibility testing (DST), molecular diagnostic assays Xpert MTB/RIF, MTBDR*plus* and MTBDR*sl*. Culture-based DST can take several weeks for results to be available, thus delaying initiation of effective treatment and contributing to the spread of drug-resistant strains. While molecular diagnostic assays are more efficient in producing results compared to culture-based DST, these assays can only detect resistance-conferring mutations in specific target regions.

In order to curb the spread of DR-TB, rapid detection and early

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treatment initiation informed by comprehensive DST results is needed. Latest developments in the field of next-generation sequencing (NGS) have given rise to a high-throughput and quick diagnostic tool for the detection of DR-TB [2], as well as understanding its epidemiology through investigating transmission chains [3]. Whole genome sequencing has recently emerged as a tool that can detect mutations known to be associated with drug resistance, but also has the potential to identify new drug resistance mutations [4]. This is particularly important with the introduction of new TB drugs into standardised DR-TB regimens, and especially with drugs for which the molecular mechanisms of resistance have not yet been fully elucidated. Moreover, the turnaround time for results with NGS is shorter when compared to phenotypic DST, with results being produced between 24–48 h depending on a sequencing platform being used and number of samples per sequencing run. This review discusses some of the challenges that are currently hampering the diagnosis and control of DR-TB in a high TB burden setting of the KwaZulu-Natal (KZN) province in South Africa and how these could be overcome using recent advancements in NGS.

2. Epidemiology of drug-resistant tuberculosis in South Africa and KwaZulu-Natal

The national TB drug resistance survey conducted by South Africa's Department of Health between 2012 and 2014 revealed that the prevalence of drug resistance in TB cases was on the rise in the country, compared to the previous survey from 2001–2002. The survey reported an estimated national overall prevalence of 4.6% for RR-TB (60% of which was MDR-TB and 40% rifampicin mono-resistance). The survey further revealed that 4.9% of MDR-TB patients had XDR-TB, with a further 16% infected with MDR-TB strains with resistance to either a fluoroquinolone or a SLID [5,6], commonly termed pre-XDR-TB. Resistance to ethionamide and pyrazinamide was estimated to be at 44.7% and 59.1% respectively in MDR-TB isolates [5]. Put together, these findings showed that one in five MDR-TB patients had significant resistance to second-line drugs, further highlighting the need for early identification and treatment of these cases.

Between 2011 and 2012, the incidence of XDR-TB in KZN was reported to have increased to 3.5 cases per 100 000 population, with the majority of districts experiencing a rise [7]. In 2017, a province-wide epidemiologic and social-network analysis for both clinical and genotypic evaluation of XDR-TB cases revealed that 69% of the cases were not due to inadequate treatment of MDR-TB (acquired resistance), but rather transmission [8]. These findings indicate that control and prevention strategies for DR-TB in the province have failed as the epidemic has been on the rise for the past decade and is likely to spread to other provinces due to migration. Currently, KZN has one of the highest XDR-TB burdens in the world, with the majority of cases attributed to transmission. Furthermore, a potential role of transportation and migration in the XDR-TB epidemic in KZN, where patients travel long distances to receive care at centralised health facilities, has been previously shown [9].

3. Challenges associated with current drug-resistant tuberculosis diagnostics

Second-line TB drugs are used to treat patients with MDR-TB, and to ensure that appropriate drugs are provided to patients, it is critical to determine as much information about drug susceptibility as possible [10]. Therefore, there's always a need for early detection of resistance to second-line drugs to better manage MDR-TB and prevent the development of XDR-TB [11].

3.1. GeneXpert technology

In order to improve the diagnosis of TB and achieve sensitivity comparable to culture-based methods, new rapid and accurate diagnostic methods are required. Substantial research has led to the development of diagnostic tests such as nucleic acid amplification tests (NAAT) that can be applied directly to sputum samples [12]. In December 2010, the WHO approved a novel rapid test for TB, the Xpert MTB/RIF assay (performed on the GeneXpert system). This cartridge-based assay is a fully automated NAAT capable of diagnosing TB and resistance to RIF (an indicator for MDR-TB) within two hours. However, it is not regarded as a point of care test due to its expensive nature and the fact that the machine can only be operated by trained personnel [13]. Due to suboptimal sensitivity in smear-negative sputum samples and occasional false identification of rifampicin resistance by Xpert MTB/RIF, Xpert MTB/RIF Ultra has recently been developed [14,15]. Xpert MTB/RIF Ultra has increased sensitivity for *M. tuberculosis* detection in smear-negative sputum, culture-positive samples and in patients with HIV compared to Xpert MTB/RIF [14,15]. Moreover, Xpert MTB/RIF Ultra has a melting temperature-based analysis, instead of real-time PCR analysis with Xpert MTB/RIF, which reduces false positive detection by allowing for the differentiation between resistance-conferring mutations and silent mutations such as Q513Q and F514F [15].

Resistance to fluoroquinolones and SLIDs, which are the backbone of MDR-TB treatment regimens, defines XDR-TB. In an effort to detect resistance to fluoroquinolones and SLIDs directly from *M. tuberculosis* sputum samples, Xie et al. (2017) assessed the accuracy of a new cartridge-based rapid molecular assay for use with the GeneXpert platform. Using phenotypic DST as a reference standard for the detection of resistance, the sensitivity of the assay was 83.3%, 90.2% and 71.1% for INH, fluoroquinolones and SLIDs, respectively, while the specificity was 94.3% for all the drugs excepts for moxifloxacin (84%). Compared to DNA sequencing, the assay had a sensitivity of 98.1% for INH, 95.8% for fluoroquinolones and 94.8% for SLIDs, with a specificity of 99.6% or greater for all drugs [16], indicating potential for its use in conjunction with Xpert Ultra as a rapid point-of-care diagnostic bundle to inform clinical decisions for patients with DR-TB. However, this assay has not been rolled out yet while DR-TB regimens are already injection-free, which is indicative of slow progress in diagnostic development.

3.2. Line probe assays

The molecular revolution in TB diagnosis began in 2008 when WHO endorsed the use of line probe assays (LPAs) for the rapid detection of MDR-TB [17]. WHO-approved Genotype MTBDR*plus* LPA is used for the rapid detection of *M. tuberculosis* and resistance to RIF and INH [18]. Performed directly on sputum samples, the MTBDR*plus* version 2.0 assay has a reported sensitivity and specificity of 98.2% and 97.8% for RIF resistance detection and 95.4% and 98.8% for the detection of INH resistance, when compared to culture-based DST [19]. Moreover, MTBDR*plus* can rapidly diagnose MDR-TB in both smear-negative and smear-positive specimens [20]. The assay has a sensitivity of 74% and 92% for the detection of *M. tuberculosis* in smear-negative and smear-positive samples, respectively [20], providing more evidence for WHO policy recommendations for the use of LPAs to detect MDR-TB [19].

MTBDR*sl* is a rapid genotypic LPA that detects resistance to fluoroquinolones and SLIDs in *M. tuberculosis* complex [10]. The assay can be performed directly in both smear-positive and smear-negative specimens without the need for culture, which eliminates delays associated with culture [11]. Compared to phenotypic DST, MTBDR*sl* has a reported sensitivity and specificity of 97% and 98% for fluoroquinolones, and 89% and 90% for SLIDs [10]. However, MTBDR*sl* can only detect mutations in *rrs* and *eis* genes, which code for amikacin, kanamycin and capreomycin resistance, and in *gyrA* and *gyrB* for fluoroquinolone resistance. Therefore, results obtained should be further confirmed by phenotypic DST and DNA sequencing [21].

Table 1
Genotypic and phenotypic drug susceptibility tests in use for specific drugs within drug-resistant tuberculosis regimens, South Africa.

Drug	Treatment regimens	Genes associated with drug resistance[a]	Genotypic test in use	Phenotypic test in use[d]	Comments
Linezolid	S/L1/L2/M	*rpl*C, *rrl*			Phenotypic DST reflex test when MTBDRsl suggests FQ or SLI resistance
Isoniazid (high dose)	S/L2/M	*kat*G, *inh*A[b], *kas*A	MTBDR*plus*[c]		Phenotypic DST reflex test when MTBDR*plus* suggests RIF resistance/INH sensitivity
Bedaquiline	S/L1/L2/M	*Rv0678*, *atp*E, *pep*Q			Phenotypic DST to be introduced in 2019
Levofloxacin	S/L1/M	*gyr*A, *gyr*B	MTBDR*sl*		Phenotypic DST reflex test when MTBDR*sl* suggests FQ sensitivity (0.25µg/L); reflex test when MTBDRsl suggests FQ or SLI resistance (0.25µg/L & 1.00µg/L)
Clofazimine	S/L1/L2	*Rv0678*, *Rv1979c*, *Rv2535c*, *ndh*, *pep*Q			Phenotypic DST to be introduced in 2019
Pyrazinamide	S/L2/M	*pnc*A, *rps*A, *pan*D			
Ethambutol	S	*emb*B, *ubi*A			
Terizidone	L1/L2/M				
Delamanid	L2/M	*fgd*1, *fbi*A, *fbi*B, *fbi*C, *ddn*			
Ethionamide	L2/M	*inh*A[b], *eth*A, *msh*A, *ndh*	MTBDR*plus*[c]		
Para-aminosalicylic acid (PAS)	L2	*thy*A, *fol*C, *rib*D			

Red shading indicates no test routinely applied in diagnostic workflow; green shading indicates test that is applied in all cases of rifampicin-resistant TB; orange shading indicates test applied in selected cases.
L1, long regimen 1; **L2**, long regimen 2; **M**, meningitis regimen; **S**, short regimen.
a Genes associated with drug resistance [22].
b *inh*A promoter and coding regions.
c Detects mutations in *inh*A promoter region (not coding region).
d Extended phenotypic DST available in cases of DR-TB treatment failure, and in cases with prior exposure to DR-TB treatment.

3.3. Limitations of targeted genotypic assays

A major setback with all current molecular diagnostic tools for DR-TB is that they only detect resistance-conferring mutations in specific target regions, therefore novel mutations and mutations outside targeted regions cannot be detected. In the 2009 national TB drug resistance survey in eSwatini, 30% of MDR-TB strains isolated were found to be carrying the *rpoB* I491F mutation which is not detected by Xpert MTB/RIF (and Xpert Ultra), a first-line diagnostic test for TB and MDR-TB in the country [22]. The silent spread of strains with the *rpoB* I491F mutation could lead to ineffective treatment [22], which poses a risk of amplification of resistance, and further transmission of these strains not only in eSwatini but also in the Southern African region due to migration. There is some recent evidence to support the international spread of these strains, as the *rpoB* I491F mutation was recently identified in 15% of a sample of INH-mono-resistant TB isolates in South Africa using a targeted PCR assay and deep sequencing, and phylogenetic analysis showed clustering of isolates with those from eSwatini [23].

In South Africa, when DR-TB is detected initially by genotypic tests (Table 1), treatment is initiated in the absence of complete and detailed drug resistance data [24]. Most importantly, these molecular diagnostic assays do not detect resistance towards new anti-TB drugs (bedaquiline, linezolid, and delamanid) that are now incorporated in DR-TB treatment regimens (Table 2) in South Africa [25]. Therefore, these standardised DR-TB treatment regimens could contain an insufficient number of active drugs and people are exposed to risks of toxicity from drugs to which their isolate has undetected resistance. Moreover, these standardised regimens are subject to change due to adverse drug reactions, and drug substitutions are often not guided by drug susceptibility data. Lastly, these assays have limited epidemiological impact as they are not routinely used to track the transmission of DR-TB and have so far played a limited role in continuous drug resistance surveillance.

4. Role of whole genome sequencing in drug-resistant tuberculosis management

Latest advancements in NGS technology have led to the development of benchtop analysers that are capable of sequencing bacterial genomes in a very short space of time [26]. The feasibility of routine diagnosis and DST for TB in a high income and low TB burden setting using whole genome sequencing has also been demonstrated, however, its application in a low income and high TB setting environment is yet to be evaluated [27]. In terms of understanding the epidemiology of TB

Table 2
Standardised treatment regimens for drug-resistant tuberculosis in South Africa.

Short regimen
4–6 LZD[a] - INH_{hd}[b] – BDQ[c] – LFX – CFZ – Z – E / 5 LFX – CFZ – Z – E
Long regimen 1
6–8 LZD – BDQ – LFX – CFZ – TRD / 12 LFX – CFZ – TRD
Long regimen 2
6–8 LZD – BDQ – DLM[d] – CFZ – TRD – Z - INH_{hd}[e] / 12 LZD – CFZ – TRD – Z - INH_{hd}[e]
Meningitis regimen[f]
6–8 LZD – BDQ – LFX – CFZ – TRD – Z – INH_{hd}[g] / 12 LFX – CFZ – TRD – Z - INH_{hd}[g]

[a] Linezolid given for two months only in short regimen.
[b] High-dose isoniazid for short and long regimens (not meningitis) – 10 mg/kg.
[c] Bedaquiline given for six months (regardless of duration of intensive phase).
[d] Para-aminosalicylic acid (PAS) can be substituted for delamanid (if not available).
[e] Ethionamide (ETO) can be substituted for high-dose isoniazid (depending on presence of *inh*A/*kat*G mutation).
[f] Delamanid can be added if available.
[g] Isoniazid dose 15 mg/kg for meningitis; can be substituted for ethionamide (depending on presence of inhA/katG mutation).

in high burden settings, whole genome sequencing has been reported to provide reliable and useful resolution for detecting transmissions, which is crucial in excluding or confirming potential transmission events defined by typing methods [3]. The use of whole genome sequencing for DR-TB management, especially in high TB burden settings, promises to provide fast high-quality diagnostics and drug resistance predictions.

Retrospectively, several studies have utilized whole genome sequencing for rapid determination of anti-TB drug resistance [26], to inform treatment for MDR-TB [28], profiling the evolution of drug-resistant mutations associated with XDR-TB [29] and personalizing therapy for XDR-TB [30,31]. Real-time whole genome sequencing provides a comprehensive and rapid diagnostic solution for DR-TB when compared with routine diagnostic methods [27]. A prospective assessment of whole genome sequencing as a rapid and affordable diagnostic tool for improving patient treatment outcomes showed 93% accuracy in species prediction and drug susceptibility determination [27], when compared with culture-based DST. Although conducted in a low TB burden and high-income setting, this assessment indicated whole genome sequencing's ability to produce essential clinical information at a reduced cost. Furthermore, current improvements in sample processing and bioinformatics analysis promise to enhance the speed and accuracy of whole genome sequencing [27].

Whole genome sequencing has the advantage of determining the complete DNA sequence of *M. tuberculosis* genome and identifying drug resistance-conferring mutations, including those associated with new TB drugs, at a single time point, thus enabling a more appropriate and accurate choice of regimen. Furthermore, drugs that are less likely to add value to the treatment outcome but will increase the risk of adverse drug reactions could be removed earlier, thus improving DR-TB outcomes. WHO-recommended regimens for DR-TB will result in resistance amplification to both old and new drugs if there's lack of knowledge on resistance to these drugs at an individual level. In an effort to improve treatment outcomes in high-resource settings such as the United Kingdom (UK), TB patients receive individualized treatment guided by individual-level DST profiles generated by whole genome sequencing [32]. Furthermore, treatment success rate of approximately 80% with individualised treatment have been reported in Netherlands, South Korea, UK and Canada [33–36]. Therefore, shorter DR-TB regimens could be used to increase access to care in resource limited settings but could also be used as a short-term solution in high-income countries that are able to offer personalized treatment through precision medicine [37].

4.1. Sequencing of *M. tuberculosis* directly from sputum samples

Several studies have shown the performance of whole genome sequencing when used for rapid detection of *M. tuberculosis* full drug resistance profile directly from sputum. In 2015, Brown et al. [38] showed for the first time the accurate and successful sequencing of *M. tuberculosis* genome directly from 24 uncultured smear-positive sputum samples using specific biotinylated RNA baits. Their findings showed a genome coverage of more than 90% and high levels of concordance between phenotypic resistance and predicted resistance when compared to culture-based and molecular methods. Known drug resistance mutations were identified within a week of sample receipt and this offers the prospect of personalized treatment which leads to improved outcomes [38]. However, the use of biotinylated RNA baits makes this method expensive and it requires special skills and machinery currently not available in most microbiological laboratories [38]. Nimmo et al. [39] applied whole genome sequencing directly from sputum through the targeted enrichment method on a sample from a patient who had been diagnosed with RIF, intermediate INH and low-level fluoroquinolone resistance using Xpert MTB/RIF and LPAs. Whole genome sequencing results revealed more *inhA* mutations consistent with high-level INH resistance and no fluoroquinolone resistance. The patient was then placed on a new regimen which was completed without relapse. In this case, whole genome sequencing directly from sputum quickly generated a complete drug resistance profile, altered the clinical management of DR-TB and showed potential for guiding personalized treatment [39].

Whole genome sequencing has a potential to transform the point of care management of DR-TB if the data extracted from drug susceptibility profiles could be augmented with strain information for surveillance [40]. Using a low-cost method of extracting DNA directly from 40 smear-positive sputum samples, Votintseva et al. [40] investigated whole genome sequencing's ability to generate diagnostic and surveillance data within a clinically relevant timeframe. DNA was successfully extracted from 39 samples and sufficient data for drug susceptibility predictions was obtained from 24 samples, with all results concordant with reference laboratory phenotypes [40]. Their findings revealed that whole genome sequencing was able to identify *M. tuberculosis*, predict drug resistance patterns and produce phylogenetic data in 44 h, further adding to the growing body of evidence about the impact sequencing could have in TB management. Moreover, whole genome sequencing directly from sputum through targeted DNA enrichment has been shown to identify *M. tuberculosis* drug resistance mutations faster than mycobacterial growth indicator tube (MGIT) culture sequencing [41].

5. Limitations and challenges to implementation of whole genome sequencing for drug-resistant tuberculosis management

One of the major stumbling blocks when it comes to the application of whole genome sequencing in clinical settings is the lack of specialized personnel to handle and analyse large sequencing data [26]. To overcome this, extensive libraries of drug resistance markers have been compiled and online tools for rapid data analyses and drug resistance prediction have been developed to progress sequencing towards real-time patient management [26]. PhyResSE and TBProfiler are two of the web-based tools that produce plain language reports and can be used by non-specialists to make drug resistance decisions from sequencing data

Table 3
Challenges and potential solutions to implementation of whole genome sequencing for the management of drug-resistant tuberculosis in South Africa.

Challenge	Solution
Low DNA yield when sequencing directly from sputum	Early MGIT culture (1–5 days) Enrichment of TB DNA signal through differential lysis, RNA baiting or multiplex PCR (Targeted NGS)
Technological complexity of NGS workflows	Switch from manual to automated extraction workflows to increase DNA yield Standardized clinical reporting
Cost of NGS	Use Targeted NGS ($50-$100 per sample) as a diagnostic tool and WGS ($150-$200 per sample) for surveillance and investigation of outbreaks and transmissions
Computer-intensive and bioinformatics-based analysis workflow	Knowledge-based and user-friendly databases e.g. ReSeqTB, PhyResSE, TB Profiler, Mykrobe Predictor
Discordance in phenotypic and genotypic drug resistance data	Intergrate phenotypic, genotypic and clinical data for diagnosis and surveillance

[42]. The presence of both host and bacterial DNA in sputum samples would result in reduced *M. tuberculosis* genome coverage and low-quality reads which would impact the detection of resistance-conferring mutations due to poor quality sequencing data. Therefore, low cost and less erroneous methods for sequencing of *M. tuberculosis* directly from sputum would have to be developed and validated. Insufficient sequencing data could be caused by primer degradation due to freeze-thaw cycles, inadequate amount of DNA template and the presence of inhibitory contaminants such as salts, phenol and ethanol. Moreover, whole genome sequencing also cannot make determinate drug resistance predictions on novel mutations that have not being fully characterized or defined [43]. Sequencing can only revolutionize and alter clinical management of DR-TB if it is applied directly on sputum samples to avoid delays that are associated with culture and generate a complete genetic drug resistance profile in a very short space of time. Alternatively, culture-dependent whole genome sequencing could be used for DR-TB surveillance and investigation of outbreaks and transmissions, whereas targeted NGS, which is cheaper and does not require culture, could be employed as a rapid diagnostic tool.

Implementing whole genome sequencing within the current diagnostic framework for DR-TB would require a standardised workflow that is widely available and consistent [2]. With recent technologies being developed, the cost of sequencing will drop, and it would become accessible for developing countries with high TB incidences. The latest Illumina iSeq 100 sequencing instrument is earmarked to decentralize sequencing for personalized patient management. With $19 000 USD ($99 000 USD for Illumina Miseq) upfront capital cost, reduced sequencing workflow hands-on time and comparable performance to the Miseq instrument, the iSeq 100 sequencer appears to be an attractive alternative for the management of DR-TB in low-middle income countries [44]. Moreover, whole genome sequencing DST has recently been shown to generate full diagnostic results in a clinically relevant turnaround time and 7% cheaper annually than MGIT culture-based DST [27]. Advantages of whole genome sequencing as a TB diagnostic tool are clear when compared to phenotypic DST and current molecular diagnostics. However, in order to successfully explore the full potential of whole genome sequencing for clinical management of DR-TB, more research is needed to fully elucidate its economic benefits and limitations in both low and high TB burden settings, as well as to build and add more evidence on the growing list of potential solutions to implementation challenges (Table 3).

6. Framework for implementation of whole genome sequencing in South Africa

Although not fully implemented, whole genome sequencing for disease management is already being performed by the National Institute for Communicable Diseases (NICD) in South Africa. In January 2016, the NICD established the Sequencing Core Facility (SCF), a fully equipped laboratory that operates Sanger and other next-generation sequencers [45]. The main aim of the SCF is to provide NGS and bioinformatics support for rapid diseases outbreak response, research activities and disease surveillance for all NICD centres [45]. The SCF was instrumental in identifying the outbreak strain during the 2017 listeriosis outbreak in South Africa. Over 500 clinical isolates of *Listeria monocytogenes* from cases were subjected to whole genome sequencing, with more than 90% found to be genetically related and belonging to the sequence type 6 (ST6) which was subsequently confirmed as the single strain causing the outbreak [46]. The National Department of Health has already adopted and invested in whole genome sequencing for DR-TB management. Whole genome sequencing is currently being performed at the National Health Laboratory Services by the Centre for Tuberculosis, a WHO-endorsed national TB reference laboratory. Currently, samples are being referred to the national reference laboratory which has enough capacity, infrastructure and personnel to perform sequencing of the highest quality. For a successful implementation of whole genome sequencing in the country, provincial hospitals would have to look into building their own infrastructure with an aim of decentralizing sequencing for improved management and control of DR-TB. Drug resistance occurring at frequencies below the detection limit of rapid molecular assays and whole genome sequencing would have to be detected with phenotypic DST.

7. Conclusion

Current phenotypic and molecular techniques for DR-TB diagnosis present a variety of gaps which are a clear indication that a new approach for rapid diagnosis is urgently needed in order to improve cure rates for DR-TB. To reduce incidence of TB and DR-TB transmission and improve treatment outcomes, the period between specimen collection, diagnosis and treatment initiation needs to be shortened. The detection of TB, MDR-TB and XDR-TB in South Africa relies on molecular diagnostic assays such as the Xpert MTB/RIF, MTBDR*plus* and MTBDR*sl*. However, these molecular assays are limited to detecting resistance to only a few first-line and second-line drugs. Therefore, early deployment of whole genome sequencing for *M. tuberculosis* clinical management would play a significant role in determining drug resistance profiles for patients and serve as an essential tool for choosing right drug combinations for improved treatment effectiveness. We therefore strongly believe that using whole genome sequencing to explore DR-TB in a clinical setting will speed up diagnosis and augment personalized treatment decisions and enable the investigation of the transmission of DR-TB, which is highly prevalent in resource limited settings such as the KZN province.

Ethical statement

This is a review article. Application for ethical clearance was not required.

Conflict of interest

Authors did not declare any conflict of interest.

References

[1] World Health Organization. Global tuberculosis report. 2018https://www.who.int/tb/publications/global_report/en/.

[2] Mahomed S, Naidoo K, Dookie N, Padayatchi N. Whole genome sequencing for the management of drug-resistant TB in low income high burden settings: challenges and implications. Tuberculosis 2017;107:137–43.

[3] Luo T, Yang C, Peng Y, Lu L, Sun G, Wu J, Jin X, Hong J, Li F, Mei J, DeRiemer K, Gao Q. Whole genome sequencing to detect recent transmission of *Mycobacterium tuberculosis* in settings with a high burden of tuberculosis. Tuberculosis 2014;94(4):434–40.

[4] Lee RS, Behr MA. The implications of whole genome sequencing in the control of tuberculosis. Therap Adv Infect Dis 2015;3(2):47–62.

[5] National Institute for Communicable Diseases. South african tuberculosis drug resistance survey. 2016. p. 2012–4http://www.nicd.ac.za/index.php/centres/centre-for-tuberculosis/population-researchsurvey/south-african-tuberculosis-drug-resistance-survey/.

[6] Ismail NA, Mvusi L, Nanoo A, Dreyer A, Omar SV, Babatunde S, Molebatsi T, van der Walt M, Adelekan A, Deyde V, Ihekweazu C, Mahdi SA. Prevalence of drug-resistant tuberculosis and imputed burden in South Africa: a national and sub-national cross-sectional survey. Lancet Infect Dis 2018;18(7):779–87.

[7] Lim JR, Gandhi NR, Mthiyane T, Mlisana K, Moodley J, Jaglal P, Ramdin N, Brust JCM, Ismail N, Rustomjee R, Shah NS. Incidence and geographic distribution of extensively drug-resistant tuberculosis in kwazulu-Natal province South Africa PLoS One 2015;10(17):e0132076.

[8] Shah NS, Auld SC, Brust JC, Mathema B, Ismail N, Moodley P, Mlisana K, Allana S, Campbell A, Mthiyane T, Morris N, Mpangase P, van der Meulen H, Omar SV, Brown TS, Narechania A, Shaskina E, Kapwata T, Kreiswirth B, Gandhi NR. Transmission of extensively drug-resistant tuberculosis in south africa. New Engl J Med 2017;376(3):243–53.

[9] Kapwata T, Morris N, Campbell A, Mthiyane T, Mpangase P, Nelson KN, Allana S, Brust JCM, Moodley P, Mlisana K, Gandhi NR, Shah NS. Spatial distribution of extensively drug-resistant tuberculosis (XDR TB) patients in KwaZulu-Natal, South Africa. PLoS One 2017;12(10):e0181797.

[10] Theron G, Peter J, Richardson M, Warren R, Dheda K, Steingart KR. GenoType® MTBDR*sl* assay for resistance to second-line anti-tuberculosis drugs. Cochrane Database Syst Rev 2016;9. cd010705.

[11] Gardee Y, Dreyer AW, Koornhof HJ, Omar SV, da Silva P, Bhyat Z, Ismail NA. Evaluation of the genotype MTBDR*sl* version 2.0 assay for second-line drug resistance detection of *Mycobacterium tuberculosis* isolates in South Africa. J. Clin. Microbiol. 2017;55:791–800.

[12] Tsara V, Serasli E, Christaki P. Problems in diagnosis and treatment of tuberculosis infection. Hippokratia 2009;13(1):20–2.

[13] World Health Organization. Policy Statement: automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin Resistance: xpert MTB/RIF system. Geneva, switzerland. 2011http://whqlibdoc.who.int/publications/2011/9789241501545_eng.pdf.

[14] Chakravorty S, Simmons AM, Rowneki M, Parmar H, Cao Y, Ryan J, Banada PP, Deshpande S, Shenai S, Gall A, Glass J, Krieswirth B, Schumacher SG, Nabeta P, Tukvadze N, Rodrigues C, Skrahina A, Tagliani E, Cirillo DM, Davidow A, Denkinger CM, Persing D, Kwiatkowski R, Jones M, Alland D. The new xpert MTB/RIF Ultra: improving detection of *mycobacterium tuberculosis* and resistance to rifampin in an assay suitable for point-of-care testing. MBio 2017;8(4):812–7.

[15] Dorman SE, Schumacher SG, Alland D, Nabeta P, Armstrong DT, King B, Hall SL, Chakravorty S, Cirillo DM, Tukvadze N, Bablishvili N, Stevens W, Scott L, Rodrigues C, Kazi MI, Joloba M, Nakiyingi L, Nicol MP, Ghebrekristos Y, Anyango I, Murithi W, Dietze R, Lyrio PR, Skrahina A, Auchynka V, Chopra KK, Hanif M, Liu X, Yuan X, Boehme CC, Ellner JJ, Denkinger CM. Xpert MTB/RIF ultra for detection of *Mycobacterium tuberculosis* and Rifampicin resistance: a prospective multicentre diagnostic accuracy study. Lancet Infect Dis 2017;18(1):76–84.

[16] Xie YL, Chakravorty S, Armstrong DT, Hall SL, Via LE, Song T, Yuan X, Mo X, Zhu H, Xu P, Gao Q, Lee M, Lee J, Smith LE, Chen RY, Joh JS, Cho Y, Liu X, Ruan X, Liang L, Dharan N, Cho SN, Barry CE3rd, Ellner JJ, Dorman SE, Alland D. Evaluation of a rapid molecular drug-susceptibility test for tuberculosis. New Engl J of Med 2017;377(11):1043–54.

[17] World Health Organization. Policy Statement: molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB). Geneva: WHO; 2008https://www.who.int/tb/features_archive/policy_statement.pdf?ua=1.

[18] Hain Lifescience GmbH. GenoType MTBDR*plus* VER 2.0: Instructions for Use, 2015. https://www.hain-lifescience.de/include_datei/kundenmodule/packungsbeilage/download.php?id=936.

[19] Nathavitharana RR, Hillemann D, Schumacher SG, Schlueter B, Ismail N, Omar SV, Sikhondze W, Havumaki J, Valli E, Boehme C, Denkinger CM. Multicenter non-inferiority evaluation of hain genotype MTBDR*plus* version 2 and nipro NTM_MDRTB line probe assays for detection of rifampin and isoniazid resistance. J Clin Microbiol 2016;54:1624–30.

[20] Seifert M, Ajbani K, Georghiou SB, Catanzaro D, Rodrigues C, Crudu V, Victor TC, Garfein RS, Catanzaro A, Rodwell TC. A performance evaluation of MTBDR*plus* version 2 for the diagnosis of multidrug-resistant tuberculosis. Int J Tubercul Lung Dis 2016;20(5):631–7.

[21] Brossier F, Guindo D, Pham A, Reibel F, Sougakoff W, Veziris N, Aubry A. Performance of the new version (V2.0) of the genotype MTBDR*sl* test for detection of resistance to second-line drugs in multidrug-resistant *Mycobacterium tuberculosis* complex strains. J Clin Microbiol 2016;54:1573–80.

[22] Sanchez-Padilla E, Merker M, Beckert P, Jochims F, Dlamini T, Kahn P, Bonnet M, Niemann S. Detection of drug-resistant tuberculosis by Xpert MTB/RIF in swaziland. New Engl J Med 2015;372(12):1181–2.

[23] Makhado NA, Matabane E, Faccin M, Pinçon C, Jouet A, Boutachkourt F, Goeminne L, Gaudin C, Maphalala G, Beckert P, Niemann S, Delvenne JC, Delmée M, Razwiedani L, Nchabeleng M, Supply P, de Jong BC, André E. Outbreak of multi-drug-resistant tuberculosis in South Africa undetected by WHO-endorsed commercial Tests: an observational study. Lancet Infect Dis 2018;18(12):1350–9.

[24] Dookie N, Rambaran S, Padayatchi N, Mahomed S, Naidoo K. Evolution of drug resistance in *mycobacterium tuberculosis*: a review on the molecular determinants of resistance and implications for personalized care. J Antimicrob Chemother 2018;73(5):1138–51.

[25] Department of Health, Republic of South Africa. Interim clinical guidance for the implementation of Injectable-free regimens for rifampicin-resistant tuberculosis in adults. Adolesc Child Pretoria 2018http://www.tbonline.info/media/uploads/documents/dr_tb_clinical_guidelines_for_rsa_september_2018.pdf.

[26] Coll F, McNerney R, Preston MD, Guerra-Assuncao JA, Warry A, Hill- Cawthorne G, Mallard K, Nair M, Miranda A, Alves A, Perdigao J, Viveiros M, Portugal I, Hasan Z, Hasan R, Glynn JR, Martin N, Pain A, Clark TG. Rapid determination of anti-tuberculosis drug resistance from whole genome sequences. Genome Med 2015;7(1):51.

[27] Pankhurst LJ, del Ojo Elias C, Votintseva AA, Walker TM, Cole K, Davies J, Fermont JM, Gascoyne-Binzi DM, Kohl TA, Kong C, Lemaitre N, Niemann S, Paul J, Rogers TR, Roycroft E, Smith EG, Supply P, Tang P, Wilcox MH, Wordsworth S, Wyllie D, Xu L, Crook DW. Rapid, Comprehensive, and affordable mycobacterial diagnosis with whole-genome sequencing: a prospective study. Lancet Respirat Med 2016;4:49–58.

[28] Witney AA, Gould KA, Arnold A, Coleman D, Delgado R, Dhillon J, Pond MJ, Pope CF, Planche TD, Stoker NG, Cosgrove CA, Butcher PD, Harrison TS, Hinds J. Clinical application of whole-genome sequencing to inform treatment for multidrug-resistant tuberculosis cases. J Clin Microbiol 2015;53(5):1473–83.

[29] Cohen KA, Abeel T, Manson McGuire A, Desjardins CA, Munsamy V, Shea TP, Walker BJ, Bantubani N, Almeida DV, Alvarado L, Chapman SB, Mvelase NR, Duffy EY, Fitzgerald MG, Govender P, Gujja S, Hamilton S, Howarth C, Larimer JD, Maharaj K, Pearson MD, Priest ME, Zeng Q, Padayatchi N, Grosset J, Young SK, Wortman J, Mlisana KP, O'Donnell MR, Birren BW, Bishai WR, Pym AS, Earl AM. Evolution of extensively drug-resistant tuberculosis over four Decades: whole genome sequencing and dating analysis of *mycobacterium tuberculosis* isolates from Kwazulu-Natal. PLoS Med. 2015;12(9). e1001880e22.

[30] Bryant JM, Harris SR, Parkhill J, Dawson R, Diacon AH, van Helden P, Pym A, Mahayiddin AA, Chuchottaworn C, Sanne IM, Louw C, Boeree MJ, Hoelscher M, McHugh TD, Bateson ALC, Hunt RD, Mwaigwisya S, Wright L, Gillespie SH, Bentley SD. Whole-Genome sequencing to establish relapse or *re*-infection with *Mycobacterium tuberculosis*: a retrospective observational study. Lancet Respirat Med 2013;1:786–92.

[31] Olaru ID, Lange C, Heyckendorf J. Personalized medicine for patients with MDR-TB. J Antimicrob Chemother 2016;71(4):852–5.

[32] National Institute for Health and Care Excellence. Tuberculosis Guidance and Guidelines. 2016https://www.nice.org.uk/guidance/ng33.

[33] Brode SK, Varadi R, McNamee J, Malek N, Stewart S, Jamieson FB, Avendano M. Multidrug-resistant tuberculosis: treatment and outcomes of 93 patients. Can Respirat J 2015;22(2):97–102.

[34] Kwak N, Kim HR, Yoo CG, Kim YW, Han SK, Yim JJ. Changes in treatment outcomes of multidrug-resistant tuberculosis. Int J Tubercul Lung Dis 2015;19:525–30.

[35] van Altena R, de Vries G, Haar CH, de Lange WC, Magis-Escurra C, van den Hof S, van Soolingen D, Boeree MJ, van der Werf TS. Highly successful treatment outcome of multidrug-resistant tuberculosis in the Netherlands, 2000–2009. Int J Tubercul and Lung Dis 2015;19:406–12.

[36] Arnold A, Cooke GS, Kon OM, Dedicoat M, Lipman M, Loyse A, Butcher PD, Ster IC, Harrison TS. Drug-Resistant TB: UK multicentre study (DRUMS): treatment, management and outcomes in London and West Midlands 2008-2014. J Infect 2017;74(3):260–71.

[37] Cox H, Hughes J, Black J, Nicol MP. Precision medicine for drug-resistant tuberculosis in high-burden countries: is individualised treatment desirable and feasible. Lancet Infect Dis 2018;18(9):282–7.

[38] Brown AC, Bryant JM, Einer-Jensen K, Holdstock J, Houniet DT, Chan JZ, Depledge DP, Nikolayevskyy V, Broda A, Stone MJ, Christiansen MT, Williams R, McAndrew MB, Tutill H, Brown J, Melzer M, Rosmarin C, McHugh TD, Shorten RJ, Drobniewski F, Speight G, Breuer J. Rapid whole genome sequencing of *Mycobacterium tuberculosis* isolates directly from clinical samples. J Clin Microbiol 2015;53(7):2230–7.

[39] Nimmo C, Doyle R, Burgess C, Williams R, Gorton R, McHugh TD, Brown M, Morris-Jones S, Booth H, Breuer J. Rapid identification of a *mycobacterium tuberculosis* full genetic drug resistance profile through whole genome sequencing directly from sputum. Int J Infect Dis 2017;62:44–6.

[40] Votintseva AA, Bradley P, Pankhurst L, del Ojo Elias C, Loose M, Nilgiriwala K, Chatterjee A, Smith EG, Sanderson N, Walker TM, Morgan MR, Wyllie DH, Walker AS, Peto TEA, Crook DW, Iqbal Z. Same-Day diagnostic and surveillance data for tuberculosis via whole-genome sequencing of direct respiratory samples. J Clin Microbiol 2017;55:1285–98.

[41] Doyle RM, Burgess C, Williams R, Gorton R, Booth H, Brown J, Bryant JM, Chan J, Creer D, Holdstock J, Kunst H, Lozewicz S, Platt G, Romero EY, Speight G, Tiberi S, Abubakar I, Lipman M, McHugh TD, Breuer J. Direct whole genome sequencing of sputum identifies drug-resistant *Mycobacterium tuberculosis* faster than MGIT culture sequencing. J Clin Microbiol 2018;56(8). e00666-18.

[42] Schleusener V, Köser CU, Beckert P, Niemann S, Feuerriegel S. *Mycobacterium tuberculosis* resistance prediction and lineage classification from genome sequencing: comparison of automated analysis tools. Sci Rep 2017;7:46327.

[43] Quan TP, Bawa Z, Foster D, Walker T, Del Ojo Elias C, Rathod P, MMM Informatics Group.. Iqbal Z, Bradley P, Mowbray J, Walker AS, Crook DW, Wyllie DH, Peto TEA, Smith EG. Evaluation of whole-genome sequencing for mycobacterial species identification and drug susceptibility testing in a clinical Setting: a large-scale prospective assessment of performance against line probe assays and phenotyping. J Clin Microbiol 2018;56(2). e01480-17.

[44] Colman RE, Mace A, Seifert M, Hetzel J, Mshaiel H, Suresh A, Lemmer D, Engelthaler DM, Catanzaro DG, Young AG, Denkinger CM, Rodwell TC. Whole-genome and targeted sequencing of drug-resistant mycobacterium tuberculosis on the iSeq100 and MiSeq: a performance, ease-of-use, and cost evaluation. PLoS Med. 2019;16(4). e1002794.

[45] National Institute for Communicable Diseases. http://www.nicd.ac.za/sequencing-core-facility/ 2019.

[46] National Institute for Communicable Diseases. Situation report on Listeriosis outbreak. 2018. South Africa.