SPECIAL ARTICLE

Diagnosis of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis: Current standards and challenges

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INTRODUCTION: The emergence of multidrug-resistant tuberculosis (MDR-TB) and, more recently, extensively drug-resistant TB (XDR-TB) is widely considered a serious threat to global TB control. Over 400,000 new cases of MDR-TB occur each year and, although their rates are currently unknown, XDR-TB cases have been detected in every country where there is capacity to detect them (including Canada).

METHODS: The present article provides a narrative overview of the various diagnostic options available for XDR-TB, including conventional tools and newer rapid tests for drug resistance. Available data suggest that automated liquid cultures are highly accurate and their use is rapidly expanding. Newly developed phenotypic tests include TK Medium (Salubris Inc, USA), microscopic-observation drug-susceptibility assay, FASTPlaque-Response bacteriophage assay (Biotec Laboratories Ltd, UK), colorimetric redox indicator methods and the microcolony method. These tests are usually cheaper but not always simple to perform, with some requiring high standards of biosafety and quality control. Among the newly developed phenotypic methods, reverse hybridization-based assays, referred to as line probe assays, represent a useful tool because of their superior accuracy and cost-effectiveness.

CONCLUSIONS: To effectively address the threats of MDR-TB and XDR-TB, global initiatives are required to scale-up culture and drug susceptibility testing capacities, especially in high-burden countries where such capacity is scarce. In parallel, efforts are needed to expand the use of novel and emerging technologies (ie, molecular diagnostics) for the rapid determination of drug resistance.

Le diagnostic de tuberculose multirésistante et de tuberculose ultrarésistante : Les normes et les défis actuels

INTRODUCTION : L'émergence de la tuberculose multirésistante (TBM) et, plus récemment, de la tuberculose ultrarésistante (TBU), est largement perçue comme une grave menace au contrôle mondial de la TB. Plus de 400 000 nouveaux cas de TBM se déclarent chaque année, et bien qu'on n'en connaisse pas le taux, on a recensé des cas de TBU dans tous les pays en mesure de les déceler (y compris le Canada).

MÉTHODOLOGIE : Le présent article contient un aperçu narratif des diverses possibilités diagnostiques de la TBU, y compris les outils classiques et les tests rapides récents de pharmacorésistance. Selon les données disponibles, les cultures liquides automatisées sont très précises, et leur utilisation prend une rapide expansion. Les nouveaux tests phénotypiques incluent le TK Medium (Salubris Inc, États-Unis), la méthode de pharmacosensibilité par observation microscopique, le dosage bactériophage FASTPlaque-Response (Biotec Laboratories Ltd, Royaume-Uni), les méthodes colorimétriques par indicateur Redox et la méthode des microcolonies. Ces tests sont généralement moins coûteux, mais pas toujours faciles à exécuter, car certains exigent des normes élevées de biosécurité et de contrôle de la qualité. Parmi les nouvelles méthodes phénotypiques, les dosages par hybridation inversée, ou méthodes par sonde en direct, constituent un outil utile en raison de leur précision supérieure et de leur rapport coût-efficacité.

CONCLUSIONS : Pour affronter la menace de la TBM et de la TBU avec efficacité, il faudra entreprendre des initiatives mondiales pour développer les capacités des tests de culture et de susceptibilité aux médicaments, notamment dans les pays où le fardeau est élevé et où les capacités sont déficientes. Parallèlement, il faudra consentir des efforts pour généraliser l'usage des technologies novatrices et émergentes (p. ex., diagnostics moléculaires), afin de déterminer rapidement la pharmacorésistance de la maladie.

The emergence of multidrug-resistant tuberculosis (MDR-TB) and, more recently, extensively drug-resistant TB (XDR-TB) is a major threat to global TB control (1-4). MDR-TB is resistant to isoniazid (INH) and rifampicin (RIF). While MDR-TB has been documented in the past (3), the term XDR-TB appeared in the literature for the first time in March 2006, in a report jointly published by the World Health Organization (WHO) and the US Centers for Disease Control and Prevention. This report described a severe form of disease caused by strains of

Mycobacterium tuberculosis which were resistant not only to INH and RIF but also to at least three of the six classes of second-line anti-TB drugs (fluoroquinolones, aminoglycosides, polypeptides, thioamides, cycloserine and para-aminosalicylic acid) (1).

Because the initial XDR-TB definition was dependent on drug susceptibility testing (DST) of second-line drugs, which is known to be unreliable, and because some forms of drug-resistant TB are more treatable than others, it was subsequently revised by the WHO XDR-TB Task Force in October 2006. XDR-TB is

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now defined as resistance to, at least, INH and RIF and, in addition, to any fluoroquinolones and to at least one of the three following injectable drugs – capreomycin, kanamycin and amikacin (1,5). These classes of drugs are the most potent and the least toxic options for second-line therapy. In addition, DST is more reliable for fluoroquinolones and injectable drugs than for other second-line drugs.

LABORATORY DIAGNOSIS OF MDR-TB AND XDR-TB

Drug-resistant TB often goes undetected and untreated in many countries. With the exception of a few developed countries, most national TB programs worldwide do not routinely provide diagnostic services based on culture and DST. The laboratory is an essential component in TB control programs, and broader access to DST is a priority for most countries. Early choice of appropriate treatment is an essential determinant of favourable outcome, and rapid determination of drug resistance can allow a customized approach to treatment early in the course of the disease and can potentially reduce morbidity, mortality and infectiousness (6).

The diagnosis of MDR-TB and XDR-TB is hampered by the absence of effective and affordable rapid diagnostic techniques for drug sensitivity. Several approaches, phenotypic and molecular, have been explored to develop rapid, reliable and accurate methods for the rapid detection of drug resistance in M tuberculosis. These methods should also be evaluated and applied in high-incidence areas.

CONVENTIONAL CULTURE-BASED METHODS

Using standardized DST procedures with conventional methods, eight to 12 weeks are required to identify drug-resistant microorganisms on solid media (ie, Lowenstein-Jensen medium). In general, such methods assess inhibition of M tuberculosis growth in the presence of antibiotics to distinguish between susceptible and resistant strains.

The proportion method allows precise determination of the proportion of resistant mutants to a certain drug; the resistance ratio method compares the resistance of an unknown strain with that of a standard laboratory strain. While relatively inexpensive and undemanding of sophisticated equipment, results usually take weeks and this is challenging; inappropriate choice of treatment regimen may result in death within weeks of initiation, such as in the case of XDR-TB (especially in HIV-infected patients). In addition, delayed identification of drug resistance results in inadequate treatment, which may generate additional drug resistance and continued transmission in the community.

LIQUID CULTURE-BASED METHODS

Automated liquid culture systems are more sensitive than solid media cultures, and they significantly reduce turnaround time. However, even with liquid cultures, two to four weeks are still needed to obtain results, and their substantially higher cost is an issue for resource-limited countries. The BACTEC 460 TB radiometric system (Becton Dickinson, USA) was considered to be a major advancement when it was introduced, but has been replaced by the Mycobacteria Growth Indicator Tube system (Becton Dickinson, USA). Several published studies have shown the excellent performance of the Mycobacteria Growth Indicator Tube system for the rapid detection of resistance to first- and second-line anti-TB drugs (7). Detection of drug resistance can be accomplished in days rather than weeks, although still constrained by high cost (equipment and consumables).

In 2007, the WHO issued policy guidance on the use of liquid TB culture, DST and rapid species identification in low-resource settings (8). The WHO policy recommends phased implementation of these systems as a part of a country-specific comprehensive plan for laboratory capacity strengthening, and addresses key issues including biosafety, customer support, staff training, maintenance of infrastructure and equipment, specimen transport and reporting of results.

NOVEL, RAPID PHENOTYPIC METHODS

Among novel, rapid phenotypic methods, the microcolony method is relatively low cost. It has been adapted for the rapid detection of drug resistance directly from sputum samples, and has been shown in early studies to be accurate for the detection of MDR-TB compared with the reference proportion method, with results available in one week (9). Newly developed phenotypic tests such as TK Medium (Salubris Inc, USA), microscopic-observation drug-susceptibility assay (MODS) and FASTPlaque-Response bateriophage assay (Biotec Laboratories Ltd, UK) are usually cheaper but not always simple to perform, with some requiring high standards of biosafety and quality control (10).

TK Medium is a novel colorimetric system that indicates growth of mycobacteria by changing the colour of the growth medium. Metabolic activity of growing mycobacteria changes the colour of the culture medium, and this allows for an early positive identification before bacterial colonies appear. TK Medium also permits susceptibility testing for drug resistance, and can allow for differentiation between M tuberculosis and nontuberculous mycobacteria. Unfortunately, there is insufficient published evidence on the field performance of this test in developing countries (10).

The MODS assay is based on the observation of the characteristic cord formation of M tuberculosis that is visualized microscopically in liquid medium with the use of an inverted microscope (11). MODS uses simple light microscopy to detect early growth of M tuberculosis as 'strings and tangles' of bacterial cells in the broth medium with or without antimicrobial drugs (for DST) (12). The agreement between MODS and the reference standard for drug susceptibility testing is 97% for INH, 100% for RIF, and 99% for INH and RIF combined (MDR). Lower values of agreement were obtained for ethambutol (95%) and streptomycin (92%). One minor disadvantage of MODS is the requirement for an inverted microscope for observation of the mycobacterial growth.

FASTPlaque-Response is a phage amplification-based test, and has been developed for direct use on sputum specimens. Drug resistance is diagnosed when M tuberculosis is detected in samples that contain the drug (ie, RIF). A recent meta-analysis of the accuracy of phage-based methods for detecting RIF resistance in M tuberculosis concluded that these assays performed on M tuberculosis culture isolates have high sensitivity, but variable and slightly lower specificity (13). Not enough evidence is available on the accuracy of these assays when performed directly on sputum samples. Safety and quality control issues related to the use of this technique should also be addressed carefully.

Several colorimetric methods have also been proposed in the past few years for the rapid detection of drug resistance in M tuberculosis. A recent systematic review and meta-analysis (14) of colorimetric redox indicator methods found evidence

of high sensitivity and high specificity for the rapid detection of MDR-TB. Colorimetric methods represent a good alternative for the rapid detection of drug resistance in laboratories with limited resources. However, these tests cannot be directly used on clinical specimens.

Overall, large multicentric studies defining the accuracy of phenotypic DST methods are still unavailable. Practical issues, such as quality controls and training requirements, have not been adequately addressed under field conditions. The application of these approaches to support individualized treatment through determination of second-line drug susceptibility profiles remains largely unexplored, implying that their application in support of individualized treatment of MDR-TB (and especially for XDR-TB) remains uncertain.

NOVEL, RAPID MOLECULAR METHODS

The identification of specific mutations responsible for drug resistance has facilitated the development of novel, rapid molecular tools for DST. The detection of RIF resistance is traditionally used as a predictor of MDR-TB – its positive predictive value is a function of the sensitivity and specificity of RIF resistance testing and the prevalence of MDR and non-MDR RIF resistance, which is highest among previously treated cases in settings with high MDR prevalence and low non-MDR RIF resistance. Molecular tools are based on nucleic acid amplification in conjunction with electrophoresis, sequencing or hybridization. Although most of the techniques were initially developed to detect drug resistance in TB complex isolates, they are being evaluated for direct detection of TB complex isolates and identification of alleles related to drug resistance in clinical specimens (such as sputum). Their potential advantage is that there is no need for growth of the organism and DST results can be determined in days rather than weeks; research suggests that they can be highly reliable.

Direct sequencing is another approach to detecting mutations, but it is an expensive and time-consuming process. Techniques, such as real-time polymerase chain reaction, that make use of wild-type primer sequences to amplify genes and enable the use of specific probes (ie, molecular beacons) to identify mutations are expensive and complicated, even if highly sensitive and specific. Reverse hybridization-based assays, referred to as line probe assays, represent a useful tool for their superior cost-effectiveness. These tests are based on the hybridization of specific probes for wild-type and mutated sequences of genes involved in drug resistance, and they show high specificity and medium/high sensitivity.

Commercially available line probe assays include the INNO-LiPA Rif. TB kit (Innogenetics, Belgium) and the GenoType MTBDR assay (Hain Lifescience, Germany). A recent meta-analysis summarized the results obtained for the INNO-LiPA Rif. TB test, and showed that this line probe assay has high sensitivity and specificity when culture isolates are used (15). The majority of studies had sensitivities of 95% or greater, and nearly all were 100% specific. The results, however, are less accurate when the test is directly applied to clinical specimens (ie, sputum). There is a paucity of data on the application of this test directly to clinical specimens.

The GenoType MTBDR test is able to detect mutations in the *rpoB* gene for RIF resistance, and the most frequent mutation at codon 315 of the *katG* gene for INH resistance, either in isolates or clinical specimens. The specificity and sensitivity of the assay for RIF resistance were nearly 100%; for INH-resistance, despite a high specificity (approximately 100%), the sensitivity of the test ranged from 70% to 90%, depending on the prevalence of the particular mutation at the *katG* locus (16,17). GenoType MTBDRplus (Hain Lifescience, Germany), an advanced version of the assay, includes probes for the identification of other mutations in the hotspot region of the *rpoB* gene for RIF resistance, and probes to detect mutations in the promoter region of the *inhA* gene involved in INH resistance. These improvements facilitate the detection of another 10% to 20% of INH-resistant cases, with an enhancement in rapid MDR-TB diagnosis.

Overall, line probe assays are accurate and useful for rapid detection of drug resistance directly in clinical specimens. However, the number of genes that can be analyzed remains limited and the test fails to distinguish insertion mutations. Furthermore, they retain a lower sensitivity among acid fast bacilli-negative samples. In general, line probe assays are expensive and require sophisticated laboratory infrastructure. Their role and utility in low-income, high-burden countries will need to be evaluated in field studies.

CONCLUSIONS

Effective control of MDR-TB and XDR-TB will require massive scaling-up of culture and DST capacity, and the expanded use of novel and rapid assays for drug resistance. Overall, molecular approaches are still insensitive for many of the mutations that allow some TB strains to remain resistant to second-line drugs due to our limited understanding of the underlying biological mechanisms. Furthermore, all genotypic tests require DNA extraction, gene amplification and detection of mutation and are, therefore, relatively expensive and demand resources and skills that are usually unavailable in most regions where rates of MDR-TB and XDR-TB are high. The challenge, therefore, is to not only develop new tools, but to also make sure that benefits of promising new tools actually reach the populations that need it most, but can least afford them.

Agencies such as the Stop TB Partnership, Foundation for Innovative New Diagnostics, the Special Programme for Research and Training in Tropical Diseases, and the WHO, are well placed to address these challenges (18). Thanks to various initiatives, the new diagnostics pipeline has rapidly expanded (10,19,20). Funding and international support for the new Global Plan to Stop TB, 2006–2015 (21) and the Retooling Task Force (22) of the Stop TB Partnership will greatly enhance the development and implementation of new tools for TB control.

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