DIAGNOSTIC MICROBIOLOGY

A clinical, microbiological and economic analysis of a national service for the rapid molecular diagnosis of tuberculosis and rifampicin resistance in *Mycobacterium tuberculosis*

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A clinical, microbiological and economic study of a national rapid molecular service for the identification of *Mycobacterium tuberculosis* and the determination of rifampicin resistance in smear-positive sputum samples (and other primary specimens) was performed. Ninety-one primary specimens, of which 55 were smear-positive sputum, were examined by molecular and conventional assays. Concordance of molecular results from smear-positive sputum specimens with tuberculosis diagnosis and rifampicin resistance by conventional analysis was 52 (94.5%) of 55 and 44 (91.7%) of 48, respectively. Concordance of molecular analysis on all primary specimens was 81 (89.0%) of 91 (diagnosis) and 55 (90.2%) of 61 (rifampicin resistance). Approximately 28 days were saved in the time to diagnosis by using the molecular assay. Hospitals can reduce the cost of inappropriate isolation of patients with risk factors for multiple drug-resistant tuberculosis (MDRTB) who subsequently are shown to have drug-sensitive tuberculosis. At one hospital potential annual savings were between £50 000 and £150 000. Of the nine MDRTB cases identified, all had a previous diagnosis of tuberculosis, 78% were born overseas, 44% were known to be non-compliant with therapy, but only one case (12.5%) was HIV positive. HIV status was not significantly different between MDRTB and drug-sensitive tuberculosis cases. Over 75% of specimens were taken while the patient was on therapy. Isolates from >50% of the MDRTB cases were resistant to three or more drugs and one was resistant to seven drugs. All patients were placed on additional therapy once the molecular result was known; this was subsequently modified based on the results of in-vitro drug susceptibility testing. All survived at least 6 months of follow-up. There was no difference in the proportion of successful cultures from smear-positive samples from patients with drug-sensitive tuberculosis or MDRTB who were on therapy. Molecular rifampicin resistance assays are reliable for diagnosis in cases with smear-positive disease.

Introduction

Infection with *Mycobacterium tuberculosis* causes nearly 8 million new cases of tuberculosis and 3 million deaths annually [1]. The worldwide extent of drug resistance is not clear, but resistance to at least one drug has been reported in nearly 10% of patients with no prior treatment; the prevalence of cases of primary isoniazid resistance, rifampicin resistance and multiple drug-resistant tuberculosis (MDRTB, i.e., cases due to strains resistant to at least isoniazid and

rifampicin) was 7.3%, 1.8% and 1.4%, respectively, in one study [2].

Drug-resistant *M. tuberculosis*, and particularly MDRTB, compromises clinical success and the effectiveness of tuberculosis control programmes because of the prolonged infectivity of the patients [3, 4]. Dramatic outbreaks of MDRTB, particularly in HIV-infected patients, in the USA [5, 6] and subsequently in Europe [7] focused international concern on the major public health threat posed by drug-resistant tuberculosis. High case-fatality rates (approaching 90% in HIV-positive individuals) were reported from the USA [3, 4, 8].

The UK is a low-incidence country for tuberculosis (c. 6000 new cases per annum; notification rate of c. 10/100 000 population), but the incidence of both drug-sensitive and -resistant tuberculosis is highly polarised geographically [9, 10]. London, for example, has a tuberculosis notification rate almost four times the national average [9]. From 1993 to 1996, cases of initial MDRTB (i.e., patients infected with MDRTB isolates who are believed not to have had a diagnosis of tuberculosis or prior therapy) increased from 0.6% to 1.6% [10].

Significant advances have been made in understanding the genetic basis of drug resistance, particularly for rifampicin; in c. 95% of rifampicin-resistant isolates, resistance is due to mutations in an 81-bp region of the *rpoB* gene [11]. Therefore, this gene is a good target for molecular genotypic diagnostic methods [12–17]. In the UK, >90% of rifampicin-resistant isolates are also resistant to isoniazid, which makes rifampicin resistance a useful surrogate marker for MDRTB.

Other studies have described the technical performance of molecular identification assays for *M. tuberculosis*, but there has been little analysis of the performance of identification and rifampicin-resistance assays on primary specimens, and none in the context of a non-trial national clinical service. Sputum smear-positive cases of pulmonary tuberculosis are the most infectious and represent the most significant public health problem. For these reasons a rapid identification service for *M. tuberculosis* with the detection of rifampicin resistance primarily for patients with smear-positive sputum (called 'Fastrack') was initiated by the Public Health Laboratory Service Mycobacterium Reference Unit in December 1996. Urgent smear-negative sputum samples, broncho-alveolar lavages (BALs) and non-pulmonary specimens (apart from cerebrospinal fluids) were also accepted for testing by special request from clinicians during the same period and were analysed in parallel for completeness.

A clinical, microbiological and economic analysis of the 'Fastrack' service was performed to determine (1) clinical/epidemiological reasons underlying specimen referral; (2) concordance of molecular results with conventional identification and rifampicin drug susceptibility testing; (3) clinical and microbiological features of MDRTB cases; and (4) the potential economic impact of the service to NHS hospitals.

Materials and methods

Clinical, microbiological and epidemiological data

Details of patient name, age, sex, ethnic group, specimen type, hospital, prior tuberculosis history, immunocompromised status, HIV positivity and clinical details (fever, weight loss, brief case history, chest

radiography, treatment) were sought prospectively in all cases from the submitting clinician and laboratory. Details of additional risk factors including contact with patients with MDRTB, alcoholism or homelessness, non-compliance with treatment, other institutional residency (prison, residential home), and immigration and country of origin were determined retrospectively from the referring physician or by review of the medical notes. The significance of differences between patients with MDRTB and drug-sensitive tuberculosis was assessed by two-tailed Fisher's exact and unpaired *t* tests as appropriate.

Processing of primary specimens and identification of mycobacterial cultures

All experimental procedures were performed in a Class 1 Biological Safety Cabinet under Category 3 biohazard containment conditions. Sputum was stained with auramine-phenol and positive smears were confirmed by Ziehl-Neelsen staining [18]. Samples were decontaminated by the NaOH/NALC method [18]. DNA was extracted from 1 ml of decontaminated specimen [17]. The remaining specimen was used to inoculate an MB/BacT rapid culture vial (Organon Teknika, Durham, NC, USA) and one Lowenstein-Jensen solid-medium slope which was incubated for at least 8 weeks or 12 weeks if the specimen was from an HIV-positive patient.

Mycobacterial cultures from primary specimens submitted to the 'Fastrack' service were identified by microscopic and macroscopic appearance, biochemical tests, DNA hybridisation (Accuprobe, Gen-Probe, San Diego, CA, USA) and thin-layer chromatography [18].

Drug susceptibility testing

The drug susceptibility of *M. tuberculosis* isolates was determined by the standard resistance ratio [18], radiometric BACTEC proportion (breakpoint concentration = 2 mg/L) [19] and microbroth dilution MIC methods [20] and these were compared with the results from molecular assays.

Preparation of DNA extracts, PCR amplification and molecular rifampicin resistance assay

A nested PCR was performed with 10 µl of extracted DNA as template and with the outer LiPA primers (LiPA1, 5'-GAGAATTCGGTCGGCGAGCTGATCC-3'; LiPA2, 5'-CGAAGCTTGACCCGCGCGTACA CC-3') for the first round of amplification: 30 cycles of 95°C for 60 s, 58°C for 30 s and 72°C for 90 s. One µl of the first round product was transferred to a 40-µl reaction mixture containing the inner LiPA primers (LiPA3, 5'-GGTCGGCATGTGCGGATGG-3'; LiPA4, GCACGTCGCGGACCTCCAGC) for the second round of amplification: 30 cycles of 95°C for 20 s, 65°C for 30 s and 72°C for 30 s [14]. The inner primers were

biotinylated at the 5' end. Each reaction mixture contained 0.2 mmol each of dATP, dCTP, dGTP, and 0.1 mmol each of dTTP and dUTP, 20 pmol of each primer and 1 unit of BIOTAQ DNA polymerase (Bioline, London). The Line Probe Assay (INNO-LiPA Rif.Tb; Innogenetics, Belgium) was used for the molecular rifampicin resistance test as described previously [14]. Extensive precautions, including the use of a three-room PCR suite, dedicated equipment and multiple negative controls were taken to avoid cross-contamination. High and low positive *M. tuberculosis* (c. 1000 and 100 genome copies) controls were included in parallel with patient samples.

Economic analysis

The time taken for routine culture, identification and susceptibility testing was determined for comparison with molecular identification and rifampicin resistance detection in all cases. The cost of inpatient bed stays and outpatient consultation visits was obtained [21]. Treatment costs were estimated conservatively as follows: daily combination formulations and six, monthly outpatient (OPD) visits for patients with drug-sensitive tuberculosis; the NHS billable cost of daily treatment for a minimum of 18 months for patients with MDRTB were calculated [3, 4, 22–24] assuming at least one inpatient stay of up to 56 days plus 18 OPD visits. Inpatient costs are billed as flat rates for each period of 28 days.

A more detailed analysis of the effect of rapid molecular rifampicin assays on bed usage at one London hospital was determined by review of bed occupancy data, billable costs and medical notes. The total bed days associated with occupation of negative-pressure rooms between 1 April 1997 and 31 March 1998 was calculated. The excess days and costs associated with patients subsequently shown to have drug-sensitive tuberculosis but who were inappropriately isolated in negative-pressure rooms were calculated.

Results

Clinical, microbiology and molecular results

Rapid diagnosis of tuberculosis and detection of rifampicin resistance were performed on smear-positive sputum samples from 55 patients. The clinical, demographic and microbiological characteristics of all sputum smear-positive patients are given in Table 1. Of factors previously associated with drug resistance, 32 patients had a previous diagnosis of tuberculosis, five were HIV-positive, 21 were failing to improve on therapy, two were prisoners, two were contacts of MDRTB cases and 12 were immigrants from countries with a high incidence of tuberculosis overall or of resistant *M. tuberculosis*. Clinical data were reported on 43 (78.1%) of the smear-positive patients. In 33 (76.7%) of these cases, at least one of the following symptoms or signs was reported: fever, cough, weight loss, night sweats, haemoptysis, lymphadenopathy or a strong Mantoux reaction with an abnormal chest radiograph or two of the signs or symptoms alone. MDRTB was grown from sputum specimens from nine patients (eight smear-positive, one smear-negative). Overall, specimens from c. 1.7% of all bacteriologically confirmed cases in the UK and 1% of all reported notifications nationally were examined by the molecular rifampicin resistance test.

Molecular identification results and factors associated with MDRTB

The comparison of rapid tuberculosis and rifampicin resistance results with conventional identification and drug susceptibility testing is shown in Table 2. The concordance of molecular tuberculosis diagnosis and rifampicin resistance determination with conventional methods was 94.5% and 91.7%, respectively.

Although the 'Fastrack' molecular rifampicin resistance service is specifically targeted to smear-positive sputum samples, clinicians submitted other specimens by special request. The concordance with tuberculosis diagnosis for smear-negative sputum samples was only

Table 1. Clinical, demographic and microbiological characteristics of all patients from whom smear-positive sputum samples were received (n = 55) by the 'Fastrack' molecular rifampicin resistance service

Patient or microbiological characteristic	Male patients	Female patients	Total
Number of cases (percent of total)	39 (70.9)	16 (29.1)	55 (100)
Drug-sensitive tuberculosis cases (percent of total)	27 (69.2)	11 (68.8)	38 (69.0)
MDRTB cases (percent of total)	5 (12.8)	3 (18.8)	8 (14.5)
Mean age in years (n)	43.1 SEM 2.7 (39)	42.7 SEM 3.2 (15)	42.9 SEM 2.1 (54)
Prior tuberculosis/n (%)	23/36 (63.9)	9/14 (64.3)	32/50 (64)
HIV-positive/HIV status known (%)	3/19 (15.8)	2/6 (33.3)	5/25 (20)
All immunocompromised/n (%)	5/21 (23.8)	3/7 (42.9)	8/28 (28.6)
Sample taken on therapy/n (%)	16/21 (76.2)	5/6 (83.3)	21/27 (77.7)
Other mycobacterial cultures isolated	2 (<i>M. avium</i> -intracellulare; <i>M. mageritense</i>)	0	2

n, number of cases for which data were known.

Table 2. 'Fastrack' molecular tuberculosis identification and rifampicin detection results

Specimen	Tuberculosis identification: number correct/total examined	Concordance (%)	Rifampicin resistance: number correct/total examined	Concordance (%)	Mean days saved*
All primary specimens	81/91	89.0	55/61	90.2	28.0 SEM 3.3
Smear-positive sputum	52/55	94.5	44/48	91.7	27.8 SEM 3.4

*Total represents days saved by comparison with conventional culture for which rifampicin susceptibility was determined conventionally.

70% (7 of 10), but was 80.6% (29 of 36) for all sputum specimens, 84.6% (11 of 13) for BALs, 83.3% (5 of 6) for pleural fluids, 100% (2 of 2) for lymph nodes and 80.0% (4 of 5) for biopsies. For rifampicin resistance the concordance was only 50% (2 of 4) for smear-negative sputa, but was 80% (4 of 5) for BALs, 100% (2 of 2) for pleural fluids and 100% (1 of 1) for lymph node.

Microbiological, clinical and risk factors underlying MDRTB cases

Table 3 summarises the principal clinical details of all nine (eight smear-positive, one smear-negative) MDRTB cases. All MDRTB cases had a previous

Table 3. Clinical and microbiological data on all MDRTB cases (n = 9)

Clinical or microbiological factor	Number (%) of cases with factor/number of cases for which data were known
Male gender	6/9 (66.7)
Previous tuberculosis	9/9 (100)
Sample taken on therapy	7/9 (77.8)
Born overseas	7/9 (77.8)
HIV-positive	1/8 (12.5)
MDRTB (isoniazid + rifampicin only)	4/9 (44.4)
MDRTB (3 drugs)	1/9 (11.1)
MDRTB (4+ drugs)	4/9 (44.4)
Non-compliant	4/9 (44.4)
Alive at 6 months	9/9 (100)

diagnosis of tuberculosis and >75% were born overseas. Nearly half were known to be non-compliant with therapy. Only one case was HIV-positive of eight cases in which the HIV status was known. Isolates from >50% of the MDRTB cases were resistant to three or more drugs and one was resistant to seven drugs.

Table 4 compares potential microbiological and epidemiological risk factors between patients with culture-proven MDRTB and drug-sensitive tuberculosis in sputum smear-positive cases. More smear-positive sputum samples came from men (70.9%), although there was no significant difference between the proportion of males among MDRTB and drug-sensitive tuberculosis cases. The number of cases born overseas in the MDRTB and drug-sensitive tuberculosis groups was significantly different, but was more likely to have been reported in the MDRTB group. No significant difference was seen in the proportion of HIV-positive or immunocompromised individuals amongst patients with MDRTB or drug-sensitive tuberculosis, although HIV status was known in only 54% of smear-positive cases. It was more likely to be known in the MDRTB group.

The time taken to culture, identify and determine rifampicin resistance (and resistance to other first-line drugs) in samples from all MDRTB cases was significantly longer than for all drug-sensitive tuberculosis cases overall (mean = 55 SEM 14.8 days versus 31 SEM 2.5 days; $t = 2.83$, $df = 44$). There was no

Table 4. Microbiological and clinical risk factors associated with smear-positive cases

Factor	Number with factor/number of cases in which data are known (%)		
	MDRTB cases	Drug-sensitive cases	Significant difference (p value)
Male sex	5/8 (62.5)	28/38 (73.7)	No (0.6689)
Prior tuberculosis	8/8 (100)	19/34 (55.9)	Yes (0.0352)
			OR = 13.51 (CI 0.722–253.0)
HIV-positive	1/7 (14.3)	3/15 (20.0)	No (1.000)
Born overseas	7/8 (87.5)	5/23 (21.7)	Yes (0.002)
			OR = 25.2 (CI 2.5–256)
Age	5 SD 4.0	45.4 SD 2.6	No ($t = 1.726$; $df = 44$)
Samples taken while on therapy	6/8 (75)	16/38 (42.1)	No (0.1278)
Successful cultures from samples taken on therapy	6/7 (85.7)	11/16 (68.8)	No (0.6214)
Days to culture, TB ID&R	55 SEM 14.8 (8)*	31 SEM 2.5 (38)	Yes ($t = 2.827$; $df = 44$)
Days to culture, TB ID&R of samples taken on therapy	47.5 SEM 14.3 (6)	36 SEM 5.1 (11)	No ($t = 0.927$; $df = 15$)

*8 MDRTB, 38 drug-sensitive cases; TB ID&R, days taken to culture, identify M. tuberculosis and determine rifampicin determination in all specimens or in samples known to have been taken on therapy.

significant difference between the days taken to culture and identify MDRTB and drug-sensitive tuberculosis cases (mean = 47.5 days SEM 14.3 days versus 36 SEM 5.1 days; $t = 0.928$; $df = 15$) when samples from cases known to be on therapy were considered. The number of days taken to culture specimens from MDRTB patients on therapy appears shorter than the time taken for the whole MDRTB group, but although the treatment status of 25% of the MDRTB patients was not defined it is highly likely that they were also on supervised chemotherapy at the time the sample was taken.

Discrepant results

Table 5 lists the discrepant rifampicin resistance results, i.e., those individuals from whom sputum was obtained in which the molecular rifampicin resistance result did not agree with that from the conventional assay.

In case I, the rapid molecular assay correctly identified the presence of *M. tuberculosis* in the sputum but did not detect mutations associated with rifampicin resistance. The resulting culture was also correctly identified as *M. tuberculosis*, but was rifampicin-sensitive by molecular assay. Conventional culture-based drug susceptibility testing showed the isolate to be low-level resistant at two reference centres but sensitive at a third. The patient was from Eastern Europe, gave no prior history of tuberculosis and had significant cavitary disease. He was cured on quadruple therapy supplemented with additional agents.

In case II, DNA sequencing confirmed the presence of a mutation that would normally confer resistance (data not shown). However, testing by the resistance ratio, radiometric BACTEC and microbroth dilution MIC assays indicated rifampicin sensitivity. As mutations were clearly present it is possible that compensatory mutations elsewhere in the gene restored at least partial function.

Case IV was a male diabetic who had been admitted with drug-sensitive tuberculosis in 1996 and later admitted to the same institution as a relapsed case in 1997. Isoniazid resistance was identified in 1997 and the patient was subsequently admitted for directly

observed therapy because of poor compliance. Although rifampicin resistance was identified on conventional testing, a low number of colonies was seen, i.e., borderline rifampicin resistance. Subsequently, restriction fragment-length polymorphism analysis with the insertion sequence IS6110 indicated that this isolate and that from case V were the same (data not shown), i.e., undiagnosed nosocomial transmission had occurred. The sputum and isolate in case V gave the same results on molecular analysis as for case IV and came from a second individual at the same institution. This patient was also a male with an isoniazid-resistant isolate identified 3 months previously who was admitted for non-compliance with his therapy.

Economic analysis

An analysis was performed of the additional costs associated with case management in negative-pressure room isolation of potential MDRTB cases over 1 year at one major London hospital (Table 6). Between 1 April 1997 and 31 March 1998, there were 15 patient episodes involving isolation in negative-pressure rooms for a total of 449 days. Two HIV-positive patients had MDRTB and were isolated appropriately for 96 days, indicating that 353 days of negative-pressure-based care was used inappropriately. If it is assumed, conservatively, that for each episode it would have taken 5 days to identify tuberculosis and determine rifampicin susceptibility by molecular analysis, then 75 days (5×15) would be subtracted from the total leaving 278 days of 'wasted extra cost'. The savings that one institution, albeit in an area of high TB incidence, might make was a maximum of $278 \times £552 = £153\,456$. The floor area occupied by a negative-pressure room for one patient at this institution is approximately twice that of a conventional thoracic bed, so it might be considered that the minimum saving would be that of the 'extra bed' or £198 per day = £55 044. Therefore, one hospital could have saved between £50 000 and £150 000 (Table 6).

Discussion

A national molecular service for the identification of *M. tuberculosis* and the detection of rifampicin resistance in smear-positive sputum was instituted after

Table 5. Analysis of discrepant rifampicin-resistant results in sputum samples compared to conventional analysis

Specimen type	Case number and notes
Smear-positive sputum	I. Culture and sputum examined; conflicting conventional rifampicin result (see text)
	II. Resistant tuberculosis by rapid assay, sensitive by conventional assay. Previous therapy, poor compliance; recovery on quadruple therapy (see text)
	III. Sensitive tuberculosis by rapid assay, resistant by conventional assay; MDRTB isolate
	IV. Sensitive tuberculosis by rapid assay, resistant by conventional assay; MDRTB; this isolate and that from case V were the same, i.e., nosocomial transmission (see text)
Smear-negative sputum	V. Sensitive tuberculosis by rapid assay, resistant by conventional assay; linked to case IV. Subsequent culture had S2 band mutation by InnoLipa, i.e., rifampicin-resistant
	VI. Resistant tuberculosis by rapid assay, sensitive tuberculosis by conventional assay

Table 6. Potential annual savings at one London hospital by rapid identification of MDRTB cases and reduction in inappropriate negative-pressure room isolation

Number of inpatient isolation episodes in NPR rooms (1997–1998)	15
Total number of NPR isolation days*	449 days
Number of 'correct' NPR isolation days for MDRTB (2 MDRTB cases)*	96 days
Inappropriate NPR isolation days (449 – 96), i.e., days in which drug-sensitive cases were isolated in NPR	353 days
Assume 5 days to rapidly identify <i>M. tuberculosis</i> and rifampicin resistance per episode (15 × 5)	75
Final wasted drug-sensitive tuberculosis days spent in NPR (353 – 75)	278
Excess daily cost (NPR day cost – daily thoracic bed day cost) [†] (£750 – 198)	£552
(a) Maximum saving (278 × £552)	£153 456
(b) Using 'extra bed' floor space calculation (see text) (278 × £198)	£55 044
(c) Individual test costs for analysis of 2 specimens per NPR episode (15 × 2 × £120)	£3600
(d) Final cost saving (a – c) or (b – c)	£149 856 or £51 444
(e) NHS costs for ambulatory care drug-sensitive cases (70 kg; daily quadruple therapy 2 months; 4 months R + I; 6 OPD visits)	£1200
(f) Minimum NHS billed MDRTB costs (up to 56 days as inpatient; 18 OPD; Ci + Pz + E daily therapy for 18 months ambulatory care) [‡]	£10 038

NPR, negative-pressure isolation; OPD, outpatient department; R, rifampicin; I, isoniazid; Ci, ciprofloxacin; Pz, pyrazinamide; E, ethambutol.

*Isolation in accordance with published guidelines [23, 24].

[†]Costs derived [22].

[‡]These are billed NHS inpatient (£2142 up to 28 days) and outpatient (£156 per visit) costs not actual daily costs plus drug costs [25] for three drugs for minimum 18 months therapy.

a pilot research study. Other primary specimens were examined during this period in response to clinical request.

Four studies in the USA [3, 4, 25–27] had indicated a reduced mortality (even among HIV-positive individuals) as a result of early MDRTB detection and the institution of therapy based on in-vitro drug susceptibility testing. For example, in a study of 38 mainly HIV-positive MDRTB patients, an overall response rate of 50% was seen with a median survival of 315 days [25]. First-line drug susceptibilities were available in a median of 30 days. Therefore, the rationale for a national molecular rifampicin resistance service would be to improve patient survival by the early identification of MDRTB. This would lead to the immediate addition of further anti-tuberculosis drugs to the patient's regimen which would subsequently be tailored on the basis of in-vitro drug susceptibility testing. All nine MDRTB patients identified in this study were placed on additional drugs immediately and therapy was modified subsequently on the basis of in-vitro drug susceptibility testing. All survived at least 6 months of follow-up.

More samples were taken while the patient was on drug therapy among MDRTB cases than drug-sensitive cases, but this was not statistically significant. Where declared, >75% of smear-positive sputum samples were taken while the patient was on therapy. Conventionally, the culture of samples taken from patients on therapy is believed to be of little value, particularly in drug-sensitive cases, as bacteria would not be viable. Nevertheless, culture was successful in samples taken from 11 patients with drug-sensitive tuberculosis as well as MDRTB cases on therapy. There were several features in the group of patients with drug-sensitive tuberculosis that indicated compliance problems that would make successful culture more likely: seven had had a prior diagnosis of tuberculosis, nine had failed to improve on therapy, three were alcoholic and six were

non-compliant with treatment, two of whom were subsequently detained in hospital to improve compliance.

In general, culture is more likely to be successful in those patients taking no medication, taking their medication erratically or in drug-resistant cases, but using the most advanced culture systems it is possible to obtain positive cultures from many samples taken on therapy, as shown here.

Rapid identification of tuberculosis in smear-positive specimens has been performed by different molecular amplification techniques. There are clear benefits in also determining rifampicin resistance and a survey of UK clinical laboratories indicated that a national service to determine resistance in smear-positive sputum samples was needed [28].

In this study, results were compared with the prospectively determined 'gold standard' of bacteriological culture even where there were clear reasons for discrepant results. 'False negatives' can be explained in that amplification-based assays are not as sensitive as culture methods, which is supported here in that only 70% of smear-negative culture-positive sputum samples were identified as tuberculosis, which is in line with other published studies. 'False-positives' (i.e., PCR products with a negative culture) were probably due to specimens which were taken whilst the patient was on treatment or loss of viability during specimen decontamination. For example, cases IV and V were on chemotherapy when samples were taken. The decontamination process accounts for the inability to culture a small percentage of smear-positive sputum specimens and laboratories aim to limit the loss of viable cultures through the decontamination process to c. 2%.

All the sputum specimens for which there were discrepant rifampicin-resistant results were correctly identified as *M. tuberculosis*. Most discrepant results

were due to low-level resistance isolates. Interestingly, a single MDRTB type was demonstrated by IS6110 DNA fingerprinting (data not shown) to be responsible for cases IV and V, identifying a small, previously unknown, nosocomial tuberculosis outbreak.

Against these encouraging results, between 5% and 10% of genuine rifampicin resistance might not be detected by this genotypic method [11, 15, 17]. It is likely that the full cost savings could not be achieved until the accuracy of these methods can be improved. Although the cost-effectiveness of rapid molecular tuberculosis identification systems has been debated [29–31], there has been limited analysis of the likely benefits of a molecular rifampicin resistance service in the context of a state-funded health care service. The American Thoracic Society consensus statement on rapid diagnostic tests for the diagnosis of tuberculosis indicated that, although these tests had been evaluated in clinical laboratories, they had not been examined for their utility in 'routine clinical use or public health settings' [31].

In the UK, mycobacterial cultures are identified and drug resistance determined by the Public Health Laboratory Service (PHLS) and Scottish Mycobacteria Reference Laboratory at no cost to hospital laboratories, but the examination of primary samples will in general incur a charge. In practice, therefore, those requesting molecular rifampicin resistance tests would have made an informal cost-benefit analysis based on the charge for the test and potential costs saved or clinical benefits accrued by an earlier result. During this period, 9 (9.9%) of 91 specimens referred for rapid rifampicin resistance analysis by clinicians were MDRTB, indicating that specimens were being selected successfully on the basis of putative risk factors, because the UK national MDRTB incidence rate was <2% [10].

A practical consensus might consider three cost-effective areas of use for molecular diagnosis in tuberculosis: (1) the identification of infections as *M. tuberculosis* and not *M. avium-intracellulare* (MAI) in immunocompromised patients where either diagnosis is likely; (2) the diagnosis of drug-sensitive tuberculosis (or MDRTB) where the consequences of a missed diagnosis would be severe as in tuberculosis meningitis; and (3) to determine appropriate levels of patient isolation. Institutions would benefit as no infection control measures would be necessary if the 'smear-positive case' was due to infection with MAI rather than *M. tuberculosis*. Similarly, high costs are incurred in treating potential MDRTB patients in accordance with UK guidelines [22, 23], which require treatment in negative-pressure isolation facilities; rapid analysis would be valuable in determining the need for costly negative-pressure isolation. Molecular rifampicin resistance assays can reduce the excess costs associated with the isolation of patients with risk factors for MDRTB who subsequently are shown to have drug-

sensitive tuberculosis, as these patients could be treated in cheaper ward-based side-rooms. Similarly the scope of contact tracing will be influenced by these tests.

The actual test costs included rapid culture and the numerous controls essential to the identification of cross-contamination and the maintenance of an accurate quality service. Nevertheless, significant institutional savings could be made. The cost of molecular rifampicin assays represents <10% and 1% of the total NHS billing cost (i.e., the cost that the NHS uses in extracontractual referrals to other NHS hospitals rather than the true 'real' cost, which is higher) of ambulatory treatment for drug-sensitive tuberculosis and for MDRTB treatment, respectively, in the UK (Table 6).

The high infrastructure costs needed to perform molecular rifampicin resistance assays reliably with the ability to culture isolates, confirm molecular rifampicin resistance and identify susceptibility to other anti-tuberculosis drugs would support a centralised model to reduce the unit cost of each test [32], in the context of a country with a low incidence of rifampicin resistance and MDRTB. Adequate staffing and infrastructure with the use of multiple appropriate controls to identify cross-contamination are essential, as attempts to perform these assays without proper facilities and safeguards leads to significant error. Noordhoek et al. [33] sent 200 BCG-spiked specimens as part of a blinded study to seven laboratories which were skilled in PCR-based detection methods. All laboratories were able to detect 10^7 organisms/ml, but only two were able to detect 10^4 organisms/ml. False positive rates of 0–77% were detected. The best results were obtained when PCR-based methods were performed in large clinical laboratories with sufficient space to separate preparation and detection areas, with the ability to culture specimens to audit PCR performance and to perform DNA fingerprinting to detect contamination.

Clinically, early diagnosis of rifampicin resistance and MDRTB permits the early instigation of appropriate therapy, as discussed earlier. Nevertheless, in the UK, 380 000 specimens are taken annually from patients in whom tuberculosis is a differential diagnosis, but they yield only c. 3500 bacteriologically proven cases [10, 28]. Therefore, use of these tests for screening would be inappropriate, but it would be appropriate to apply these tests to infectious patients with risk factors for drug resistance including contact with MDRTB cases, patients with prior tuberculosis and those born overseas in high incidence countries, as well as at risk immunocompromised individuals.

Nationally, the savings that might be made would depend on the local incidence of tuberculosis and MDRTB, in particular, and the availability of negative-pressure isolation. There are 425 NHS [34] provider hospital trusts in England and Wales and if it is assumed that a single day of negative-pressure isolation

was saved by each institution, the cost of a centrally organised service would be provided for.

The performance of molecular rifampicin resistance assays is technically feasible, and in a low-incidence country with adequate transport infrastructure, a centralised model is an effective one. Areas such as London with a higher incidence of tuberculosis and a higher rate of drug resistance can benefit from a central model targeted at the most infectious cases and those which are likely to be rifampicin-resistant (including samples from patients with prior tuberculosis, birth in high incidence countries, contact with MDRTB cases and poor compliance). In this, smear-positive specimens are referred to a single centre for rapid culture and analysis of rifampicin and isoniazid resistance with molecular rifampicin assays applied to those specimens with risk factors for drug resistance as described above [32].

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