Multidrug resistance after inappropriate tuberculosis treatment: A meta-analysis

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Word count abstract: 191
Word count text: 3,301

Running Head: Risk for MDR-TB after inappropriate TB regimens

Abstract

We conducted a systematic review and meta-analysis to assess the evidence for the postulation that inappropriate tuberculosis (TB) regimens are a risk for development of multidrug resistant tuberculosis (MDR-TB).

MEDLINE, EMBASE and other databases were searched for relevant articles in January 2011. Cohort studies including TB patients that received treatment were selected and data on treatment regimen, drug susceptibility testing results and genotyping results before treatment and at failure or relapse were abstracted from the included articles.

Four studies were included in the systematic review, 2 could be included in the meta-analysis. In these 2 studies the risk for development of MDR-TB in patients who failed treatment and used an inappropriate treatment regimen was 27-fold increased (26.7, 95% Confidence Interval 5.0-141.7) compared to individuals who received an appropriate treatment regimen.

This review provides evidence for the general opinion that development of MDR-TB can be caused by treatment that is inadequate, given the drug susceptibility pattern of the *Mycobacterium tuberculosis* bacilli. It should be noted that only 2 studies provided data for the meta-analysis. The information can be used to advocate for adequate treatment for patients based on drug resistance profiles.

Introduction

Multidrug resistance remains a threat to tuberculosis (TB) control (1). It is generally accepted that the development of drug resistance and multidrug resistant TB (MDR-TB) is caused by inadequate treatment, i.e. regimens with inadequate number of drugs to which the bacilli are susceptible, inadequate dose or dosing frequency, inadequate quality of the drugs, or inadequate adherence to the regimen. The aim of this review was to assess the evidence for this hypothesis. As new anti-TB drugs become available, it is important to, already at an early stage, assess the best process for their introduction and use in TB regimens. If the hypothesis holds, it will add to and strengthen the evidence that the introduction of new TB drugs in TB treatment regimens needs to be done with great care to prevent the development of resistance against new TB drugs.

Assessing whether drug resistance develops when inadequate treatment is provided requires studies that provide information about drug susceptibility testing (DST) before the start of treatment, the treatment regimen taken by each individual in the study, drug susceptibility testing of specimens obtained from individuals that fail treatment or that relapse, and genotyping information of the initial strain (collected before treatment) and of the strain that is present at failure or relapse for patients that fail treatment or relapse.

Data from low and high prevalence areas show that patients can be re-infected with a new strain of TB during treatment or after successful treatment (2;3). Since patients might be re-infected with a resistant strain, 'acquired' drug-resistance can only be diagnosed if the possibility of re-infection is excluded. Re-infection can only be excluded by genotyping the strain that caused the initial episode and the strain that is present in the sample taken at failure or recurrence. The method most frequently used to collect information about the genotype is Restriction Fragment Length Polymorphism Analysis (RFLP) (4). Van Embden et al. (4) first proposed this standard methodology for strain identification in 1993. Other methods are variable number of tandem repeats typing (VNTR) (5) or spoligotyping (6).

We conducted a systematic review and meta-analysis according to the Cochrane Handbook for Systematic Reviews and the PRISMA statement (7;8) to assess the risk for development of multidrug drug-resistant TB after the use of inappropriate TB regimens.

Methods

Search strategy

To identify relevant studies we conducted a literature search in the bibliographic databases MEDLINE and EMBASE in January 2011. We searched for guidelines in the National Guideline Clearinghouse, and the NICE and SIGN databases. Abstracts of conference proceedings were sought in BIOSIS. Reviews and guidelines were searched for in the TRIP database. The WHO International Clinical Trials Registry Platform was evaluated for ongoing trials that might provide relevant data. Key words used in the search were determined in collaboration with the clinical librarian of the Dutch Cochrane Centre and included "Tuberculosis" OR "TB" OR "Mycobacterium" AND for TB treatment "Prescriptions" OR "Treatment regimen" OR "Combination treatment" OR "Treatment strategy /-ies" OR "Drug supply" OR "Standard treatment / standard regimen" OR "Inappropiate use, appropriate use, rational use, irrational use, misuse" AND for drug resistance "drug or multidrug" OR "extensive or extensively" OR "drug and resistance or resistant". We excluded case reports. The search strategy was supplemented by hand searching reference lists of identified articles and relevant review articles.

Selection of studies and quality assessment

We initially planned to include cohort studies investigating the use of inappropriate TB regimens as a risk factor for development of drug-resistance. However, none of the cohort studies identified in the search investigated the use of inappropriate TB regimens as a risk factor for development of drug-resistance. Therefore, we widened our selection and inclusion criteria to cohort studies that provided treatment to non MDR-TB patients and measured drug-resistance and genotype of the isolated *M. tuberculosis* bacilli before the start of treatment and drug-resistance and genotype in failure and/or recurrent TB cases. Only studies that included TB patients meeting either the World Health Organization (WHO) definitions for 'definite case' (9), or meeting the 'possible', 'probable' or 'definite' case definition as published by the European Commission in 2008 (10) were included. Articles published in Dutch, English, French, German, Italian, Portuguese, Spanish or Swedish were included.

Studies identified by the search strategy were reviewed for eligibility based on title and abstract by one investigator (MvdW). If it could not be assessed with certainty whether there was reason for exclusion of a record the record was kept for the second selection step. Full manuscripts of the records kept based on title/abstract were assessed by one investigator (MvdW). For both steps, a 10% random sample was assessed by a second investigator (ML) and compared with the assessment of the first reviewer. Inconsistencies in assessment were discussed and disagreements resolved by consensus. A complete double selection was planned if the 10% random sample assessment revealed relevant inconsistencies.

Data extraction

One reviewer (MvdW) extracted all relevant data-items from the included studies using a data-extraction form. A second reviewer (ML) independently extracted the main results of the included studies and checked the other extracted results for a subsample of the articles. Inconsistencies were discussed to obtain consensus. The risk of bias of the individual studies was assessed by two reviewers independently using the NewCastle Ottawa Scale (11).

Data analysis and synthesis of results

For studies that were clinically and methodologically homogeneous, a meta-analysis was performed using Review Manager software (12). For other studies, the results were summarized qualitatively. If results were missing in a study (e.g. due to contamination of culture, or because the sample was not collected) attempts were made to extrapolate the missing information from the information provided in the article or by contacting the authors.

For the analysis, appropriate treatment regimens were defined for TB patients with different drug-resistance patterns. The WHO tuberculosis treatment guidelines were a source for defining the number and type of effective drugs required for the initial treatment phase and for the continuation phase (Table 1) (9;13). Treatment regimens were considered appropriate if the patient had disease due to a pan-susceptible strain and the regimen contained, isoniazid (H) and rifampicin (R) and two other drugs in the intensive phase, and HR in the continuation phase. If the patient had disease caused by a strain mono-resistant to H the intensive phase needed to contain HR and two other drugs and in the continuation phase, HRE. Patients with non MDR-TB that were susceptible to R needed a regimen with at least 3 drugs to which the strain is susceptible in both phases and patients with non MDR-TB and resistance to R needed a regimen that included at least four drugs to which the strain was susceptible. All other regimens were considered inappropriate.

Definitions used are provided in Box 1 (9;14). In this study, acquired MDR-TB was defined as a case with an initial strain susceptible to at least isoniazid or rifampicin, which was then MDR-TB at the time of failure or disease-recurrence and with a genotype pattern identical to the initial strain at time of first diagnosis.

The quality of the evidence was assessed using the GRADE approach (15).

Results

Study selection

Of the 701 records identified from the MEDLINE and EMBASE searches, 81 were kept for evaluation of the full manuscript. Of these records, two studies fulfilled the eligibility criteria (16;17). Figure 1, shows why records were excluded based on the assessment of title/abstract and full manuscripts. Three relevant reviews were identified in the MEDLINE and EMBASE search (18-20).

The National Guideline Clearinghouse, NICE, SIGN, and BIOSIS data bases did not provide relevant aggregated evidence. The WHO International Clinical Trials Registry Platform did not include ongoing trials that might provide relevant data. The TRIP database provided 212 systematic reviews and 220 guidelines for the search terms 'Tuberculosis' and 'Treatment'. Two additional systematic reviews were considered relevant (21;22). Checking of the reference lists of the identified reviews provided 5 potentially relevant primary studies (23-27). None of the studies could be included as it was not possible to link individual treatment information with DST and genotyping information or the treatment regimen was not mentioned in sufficient detail.

Hand searching reference lists of identified articles provided 2 additional papers that fulfilled the eligibility criteria (28;29).

Description of the included studies

All four included studies were cohort studies (Table 2 and 3). They included 233 to 2901 TB patients at the start of treatment that were followed-up until the end of treatment, to identify failure cases (16;28), or followed-up for >1.5-3 years after treatment, to identify cases that had recurrent TB (17;29).

The included studies did not provide sufficient detailed information about dose or dosing frequency, quality of the drugs, or adherence to the regimen, to assess the association of these factors with the development of MDR-TB.

Sonnenberg et al. (29) included TB patients with an episode of tuberculosis that was proven by culture. Quy et al. (17) included new smear-positive tuberculosis patients (either two sputum smears with acid-fast bacilli or one positive smear and an abnormal chest X-ray consistent with tuberculosis). Cox et al. (28) included smear-positive pulmonary tuberculosis patients (at least one sputum sample reading >10 bacilli/100 fields in a sputum smear by direct microscopy). Matthys et al. (16) included patients diagnosed with TB through sputum-smear and culture. All included patients fulfilled the definition of definite TB-case according to the WHO definition (9) and the definition of the European Commission (10).

Risk of bias assessment

Table 4 presents the results of the risk of bias assessment of the four studies using the NOS Star Template. The three studies that scored 4 stars for selection all included a sample of the general population (17;28;29). None of these three studies were however considered truly representative of the average population at risk for acquiring MDR-TB. The fourth study was in a selected group, prisoners with TB who were admitted to a referral penitentiary hospital, and was therefore considered not representative of the general population (16). In all four studies, the non-exposed cohort (those who received an appropriate TB regimen) was drawn from the same community as the exposed cohort, so there was minimal risk of bias in the selection of

the non-exposed. Also, all four studies measured exposure (receiving an inappropriate TB regimen) with sufficient quality. We only selected studies that performed drug susceptibility testing before treatment so the outcome of interest (MDR-TB) was not present at the start of the study. None of the four included studies assessed differences between individuals receiving appropriate treatment and inappropriate treatment, so an assessment of the comparability of the exposed and unexposed cohort was not performed.

As mentioned in the methods section, the included studies were not designed to investigate the use of inappropriate TB regimens as a risk factor for drug-resistance. Thus, the assessment of outcome was not influenced since the authors did not look separately at the group of exposed and non-exposed TB patients. We considered a follow-up until the end of treatment adequate for identifying failure cases and a follow-up of a minimum of 1 year for identification of recurrence cases. In all four studies the follow-up was long enough for the outcome to occur.

In conclusion, study quality was moderate to high. Two studies provided data for acquired MDR-TB in failure cases (16;28). One study provided data for acquired MDR-TB in recurrence cases (29) and Quy et al. provided information for acquired MDR-TB in both failure and recurrence cases (17).

Meta-analysis

All TB patients in the study of Quy et al. received inappropriate treatment since the continuation phase consisted of isoniazid and ethambutol and not isoniazid and rifampicin (17) (Table 5). Quy et al. showed that the risk of acquiring MDR-TB was 1.5% for failure cases and 0.4% for recurrence cases, after correction for missing culture results (17). The study of Sonnenberg et al. had both patients who received an appropriate TB regimen and patients who received an inappropriate TB regimen (29). However, this study did not have any events; i.e. none of the patients presenting with MDR-TB at recurrence had a strain with an identical genotype pattern to the initial strain at time of diagnosis. As the two above studies did not have both exposed and non-exposed individuals and events, they could not be included in the meta-analysis and thus only two of the four studies were available for meta-analysis (16;28). We used a fixed-effects model for the meta-analysis due to the low number of studies. Patients who received an inappropriate treatment regimen had a 27-fold increased risk of developing MDR-TB (Risk Ratio 26.7, 95% Confidence Interval [CI] 5.0-141.7) (Figure 2). For two patients it was not clear whether they had acquired MDR-TB or whether they were re-infected with an MDR strain. One of these patients was a patient who had MDR-TB at recurrence (28), the other was diagnosed with MDR-TB at failure (16). For the main analysis we included these two cases as re-infection cases, so they were not counted as events. Including these two cases as acquired MDR-TB cases in the meta-analysis results in a slightly lower risk ratio (RR 17.7, 95% CI 4.1-77.6).

Using the GRADE approach we started with low quality of the evidence since we included observational studies. There was no need to downgrade the quality of the evidence due to study limitations, imprecision, indirectness or inconsistency. Since the information collected for this review is mainly from studies that did not assess our research question we feel that the risk for publication bias is low, and so downgrading for publication bias was also not considered necessary. The effect indentified in the meta-analysis is large (RR 26.7). Therefore, we believe that the quality of the evidence should be upgraded by 1 level, from 'low' to 'moderate'. No plausible confounding factor that would change the effect was identified. Also, there was no dose-response gradient. The overall quality of the evidence as assessed by the GRADE approach is therefore moderate. This means that we are moderately confident in the effect estimate, and that the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Discussion

In this review we identified four studies that provided information for estimating the risk of developing MDR-TB after the use of a TB regimen that is inappropriate for a patient, considering the drug-resistance pattern of the *M. tuberculosis* strain. Only two studies could be used for the meta-analysis. These two studies showed that the risk of developing MDR-TB is 27-fold increased in patients who are prescribed an inappropriate treatment regimen. This finding supports the general opinion that inadequate treatment is a risk factor for the development of drug-resistant TB and MDR-TB.

We chose to include only high quality studies in our review. Studies had to provide information about drug resistance in the initial and the recurrent episode, genotyping information of the TB strain in both episodes, and information about individual TB treatment regimens. We did come across studies on acquired drug-resistance that did not apply genotyping to exclude re-infection (30;31). One study included in our review provided information on the percentage of MDR-TB, in failure or recurrent cases, that is really acquired and not re-infection. This study by Matthys et al. showed that more than 60% (9/14) of new MDR-TB in failure cases could be attributed to re-infection. The study was conducted in a penitentiary hospital in Central Siberia where the transmission of TB and MDR-TB is believed to be high, and thus the chances to be re-infected with an MDR-TB strain as also high. A review on re-infection or relapse not focussing on MDR-TB indeed showed that the proportion of recurrences due to re-infection ranged between 0% and 100% (14). These results are in themselves not indicative, however, together with the information from the included studies it shows that an unknown proportion of the cases with 'acquired' MDR-TB in studies not performing genotyping are due to re-infection. It is therefore essential to have genotyping information to assess whether new MDR-TB is truly acquired or due to re-infection.

There are several aspects that need to be considered when interpreting DNA fingerprinting results: 1) Changes in the insertion sequence pattern due to recombination events; 2) Heterogeneity of the fingerprinting patterns in a given area; and 3) Mixed infections. Although IS*6110* fingerprint patterns of *Mycobacterium tuberculosis* isolates have a high degree of stability, changes in insertion sequence patterns are identified, especially if the time interval between which the isolates are obtained increases (32;33). Thus, two strains may be wrongly identified as different. This provides an underestimation of the number of strains that acquire drug-resistance.

In areas shown to have a low heterogeneity in fingerprinting patterns (34;35), patients have a higher chance of becoming re-infected with an identical strain. If the strain that causes re-infection is resistant to TB drugs this may wrongly lead to the conclusion that the patient acquired drug-resistance. Also, strains with a low number of *IS6110* copies (five copies or less) cannot be differentiated between each other; as the level of discrimination is low, a strain with a low copy number at failure or recurrence might be incorrectly identified as the same strain. This would overestimate the percentage of patients that acquired MDR-TB. Spoligotyping of such RFLP low copy strains can be used to further differentiate strains within identical, low-copy RFLP patterns (36).

Regarding the two studies included in our meta-analysis and the risk of overestimating the extent of acquired MDR-TB due to low heterogeneity of circulating *M. tuberculosis* strains, the following can be stated. A study (37), based on the same patient population as that described by Cox et al. (2007), identified a high degree of strain diversity among the patient population. Specifically, 152 isolates (40%) were in clusters ranging in size of 2 to 21 isolates; i.e. 60% of strains were "unique". Given that the level of strain diversity was relatively high, we believe that the risk that patients defined as having acquired MDR-TB were in fact re-infected with an

identical strain that already was MDR-TB, is small. Information about the heterogeneity of strains was not provided by Matthys et al. (2009). In an earlier study in 1999, strains from the same setting of Matthys et al. (2009) were characterized (38) and a high degree of strain homogeneity, as assessed by RFLP, was seen. The authors concluded that there was ongoing transmission of a few strains within the prison. This study was performed 8 years before the study of Matthys et al. (2009) and transmission in prisons might have changed due to TB control activities in prisons. However, we cannot rule out the possibility that some cases that were identified with acquired MDR-TB were actually due to re-infection with an MDR strain with the same RFLP pattern. If this is the case the relative risk for developing acquired MDR-TB will be lower.

Until recently, evidence of mixed infection in a single host at a single time point was infrequently observed, which probably reflected the insensitivity of DNA fingerprinting methods (39). More sensitive methods have shown that TB patients in high-incidence settings often have different *M. tuberculosis* strains in the same sputum specimen (40;41). Patients with mixed infection may thus wrongly be diagnosed with acquired drug-resistance (42). Newer genotyping methods have since become available that increase the discriminatory power of strain genotyping (43), including whole genome typing of *M. tuberculosis* (44). Use of these methods for studying acquired drug-resistance might resolve some of the challenges discussed above.

A further consideration in assessing the acquisition of drug-resistance is that drug susceptibility testing results must be interpreted with caution. A round of proficiency testing of the supranational reference laboratories showed that DST for rifampicin presented difficulties in correctly identifying resistance in these top-level laboratories (45). Also, results for ethambutol and streptomycin were poor. The studies included in our review used WHO-recommended DST methods. Two studies had all DST testing performed by a supranational reference laboratory (16;28), and one sent a random sample for quality assurance to a supranational reference laboratory (17); no discordance was observed for isoniazid and rifampicin. The study in South Africa had all testing performed by Lancet Laboratories in Johannesburg.

A limitation of cohort studies for assessing the incidence of acquired drug-resistance is that a number of included patients in the cohort may die. The cause of death could be the acquisition of MDR-TB and thus non-response to treatment. The difficulty in collecting samples from deceased patients to assess the acquisition of drug-resistance entails that these cases will commonly be missed in cohort studies on acquired drug-resistance, thereby resulting in an underestimation of the incidence of acquired resistance.

Only quality TB drugs will ensure that patients obtain the required regimen and the required dosage. Content and stability of TB drugs have been reported to be substandard (46;47) and patients receiving substandard drugs may develop drug-resistance even when prescribed an adequate regimen. The drugs used in the study of Matthys et al. were procured outside Russia and had certificates that guaranteed their quality (16). The other included studies did not provide information about the quality of the drugs.

Inadequate adherence to treatment can also be a form of inappropriate treatment and thus a risk factor for acquiring MDR-TB. Furthermore, full adherence to treatment is essential to assure cure of the patient. Adherence to treatment was not used as a criterion in this review for appropriate treatment and risk of acquired MDR-TB; however, all included studies mention how adherence to treatment was supported. All indicated that Directly Observed Treatment (DOT) was provided. Quy et al., Matthys et al., and Sonnenberg et al. provided treatment under direct observation (16;17;29). In the study of Cox et al. patients were hospitalized during the intensive phase of treatment (exposure) and received doses during the continuation

phase that were ostensibly administered under direct observation by local health care workers (28).

With regard to the applicability of the evidence, the evidence is based on the best quality studies available. However, only 2 studies could be included in the meta-analysis. If more studies become available the review and meta-analysis can be updated.

Conclusions

This review provides evidence for the general opinion that the development of MDR-TB can be caused by treatment that is inadequate, given the drug susceptibility pattern of the *Mycobacterium tuberculosis* strain. The information can be used to advocate for adequate treatment of patients based on drug-resistance profiles.

Only few cohort studies provided information on treatment regimens, drug-resistance profile before treatment and at failure or recurrence, and genotyping information at failure or recurrence. To monitor the development of acquired drug-resistance we suggest that, given sufficient resources are available, tuberculosis treatment cohort studies or surveillance systems measure both the drug-resistance profile and genotype information before start of treatment and at failure or recurrence. This would enable future, larger studies assessing the risk of resistance-development due to inappropriate treatment.

Acknowledgements

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Box 1: Definitions of (acquired) MDR-TB, recurrence, relapse and re-infection.

MDR-TB: Tuberculosis resistant to at least isoniazid and rifampicin.

Acquired MDR-TB: A case with an initial strain susceptible to at least isoniazid or rifampicin that developed MDR-TB and has a genotyping pattern identical to the strain at time of diagnosis.

Recurrence: A second episode of tuberculosis occurring after a first episode has been considered cured.

Relapse: A second episode of tuberculosis occurring after a first episode has been considered cured with the same *Mycobacterium tuberculosis* strain as the first episode.

Re-infection: A second episode of tuberculosis occurring after a first episode has been considered cured with a different *M. tuberculosis* strain as the first episode.

Table 1: Appropriate treatment regimens for tuberculosis patients with strains with certain drug resistance patterns.

Drug resistance pattern	Appropriate treatment regimen
Pan susceptible	HR and two other drugs in intensive phase and HR in the continuation phase
H	HR and two other drugs in intensive phase and HRE in the continuation phase[1]
Non MDR-TB, R susceptible	At least 3 drugs to which the strain is sensitive in the intensive and continuation phase[2]
Non MDR-TB, R resistant	At least 4 drugs to which the strain is sensitive in the intensive and continuation phase[2]

[1] Based on World Health Organization. Treatment of Tuberculosis, Fourth edition, 2009
[2] Based on World Health Organization. Guidelines for the programmatic management of drug-resistant tuberculosis. Emergency update 2008
R Rifampicin; H Isoniasid; E Ethambutol
MDR-TB: multi-drug resistant tuberculosis

Table 2: General characteristics of the study populations.

Study identification	Age in median in years or n (%)	Male, %	HIV positive, %	Type of tuberculosis (TB)	Prior TB treatment, %
Sonnenberg 2001 (29)	<30: 35 (10.7) 30-39: 163 (50.0) 40-49: 84 (25.8) ≥50: 44 (13.5)	Not reported	46.3	Culture-positive TB patients without multi-drug resistance and cured of tuberculosis	22.7
Quy (2003) (17)	15-24: 359 (12.4) 25-34: 920 (31.7) 35-44: 867 (29.9) 45-54: 399 (13.8) 55-64: 208 (7.2) ≥65: 148 (5.1)	74.2	Not reported	New smear-positive TB	0
Cox (2007) (28)	Not reported	Not reported	Not reported	Smear positive pulmonary TB	45.3
Matthys (2009) (16)	29 (range 16-66)	100	0	Culture-positive TB patients	73

Table 3: Characteristics of the study design, outcome and exposure.

Study identification	Study design	Country	Year(s) of recruitment	Setting	Inclusion	Drug susceptibility testing method	Genotyping method	Treatment adherence	Treatment regimen	Sample size
Sonnenberg 2001 (29)	Cohort study	South Africa	1995	Hospital serving four gold mines	Culture-positive TB patients without MDR and cured of tuberculosis	Bactec system or conventional proportional count method and Lowenstein Jensen medium	IS*6110* DNA fingerprinting	Direct observation of treatment	2HRZE/4HR	326[1]
Quy 2003 (17)	Cohort study	Vietnam	1996-1998	Tuberculosis control program	New smear-positive TB	Proportion method on Lowenstein-Jensen medium	IS*6110* DNA fingerprinting	Directly observed treatment	2HRZS/6HE	2901
Cox 2007 (28)	Cohort study	Uzbekistan	2001-2002	DOTS program	Smear-positive pulmonary TB patients	Lowenstein-Jensen media using proportion method or modified proportion method in Bactec 460TB	IS*6610* DNA fingerprinting and spoligotyping	Direct observation of treatment	2HRZE (with or without S)/4HR 2HRZES/1HRZE/5HRE	209 173
Matthys 2009 (16)	Cohort study	Russian Federation	1997-1998	TB treatment programme in a penitentiary hospital	Newly admitted patients diagnosed with TB through sputum smear and culture	Proportion method on Lowenstein-Jensen medium	IS*6110* DNA fingerprinting	Daily strict supervision	2HRZES/1HREZ/5HRE	233

[1] Initial pre-treatment DST result missing for 3 TB patients

TB = tuberculosis; MDR = multi-drug resistance
H: isoniasid; R Rifampicin; Z Pyrazinamide; S Streptomycin; E Ethambutol

Table 4: Risk of bias assessment using the NOS Star Template[1] for cohort studies

Study ID	Selection	Comparability	Outcome
Sonnenberg (2001)	✳✳✳✳	-[2]	✳✳✳
Quy (2003)	✳✳✳✳	-[2]	✳✳
Cox (2007)	✳✳✳✳	-[2]	✳
Matthys (2009)	✳✳✳	-[2]	✳✳✳

[1] A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome category and a maximum of two stars can be given for Comparability. For the Selection category a star is awarded if the exposed cohort is representative, if the non exposed cohort is drawn from the same community as the exposed cohort, if exposure was ascertained by secure record or by a structured interview, and if it is demonstrated that outcome of interest was not present at start of study. For Comparability one star is awarded if the study controls for the most important factor, another star can be awarded if the study controls for any additional factor. For Outcome a star is awarded if the assessment of the outcome is an independent blind assessment or by record linkage, if there was a long enough follow up for the outcomes to occur, and if there was complete follow up or if the fact that subjects were lost to follow up is unlikely to introduce bias.

[2] No comparison made between individuals receiving appropriate treatment and inappropriate treatment since this was not the interest of the authors

Table 5: Data abstracted from the included studies for meta-analysis. The patients for which it was unknown whether they acquired MDR-TB are <u>not</u> included as acquired MDR-TB.

Reference	Treatment appropriate based on DST	Number treated without MDR-TB	Failure with acquired MDR	Recurrence with acquired MDR
Sonnenberg (2001)	yes	294		0
	no	29		0
Quy (2003)	yes	0	0	0
	no	2551	38	10
Cox (2007)	yes	240	1	
	no	74	9	
Matthys (2009)	yes	127	0	
	no	62	5	

DST: Drug-susceptibility testing
MDR: Multi-drug resistance

Figure 1: Summary of literature search and study selection.

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┌─────────────────────────────────┐
│ Identified from MEDLINE, EMBASE │
│    and other databases (after   │
│   eliminating duplicates): 701  │
│             records             │
└─────────────────────────────────┘
               │
               │         ┌──────────────────────────────────────────┐
               │         │ 620 records excluded based on title and  │
               │         │ abstract:                                │
               │         │ - 110 not about tuberculosis             │
               │         │ - 8 about latent tuberculosis            │
               │────────▶│ - 297 no observational study             │
               │         │ - 58 only MDR-TB patients included       │
               │         │ - 13 no treatment provided               │
               │         │ - 60 no DST after treatment              │
               │         │ - 74 cross-sectional drug resistance     │
               │         │   survey                                 │
               │         └──────────────────────────────────────────┘
               ▼
┌─────────────────────────────────┐
│  Records retained for review of │
│       full manuscript: 81       │
└─────────────────────────────────┘
               │
               │         ┌──────────────────────────────────────────┐
               │         │ 79 records excluded based on evaluation  │
               │         │ of full manuscript:                      │
               │         │ - 6 language                             │
               │         │ - 18 no cohort study                     │
               │         │ - 23 treatment regimen is not reported   │
               │────────▶│   for individual patients                │
               │         │ - 26 drug resistance information not     │
               │         │   available before treatment and at      │
               │         │   failure or relapse                     │
               │         │ - 6 genotyping information not available │
               │         │   before treatment and at failure or     │
               │         │   relapse                                │
               │         └──────────────────────────────────────────┘
               ▼
┌─────────────────────────────────┐
│       Records included: 2       │
└─────────────────────────────────┘
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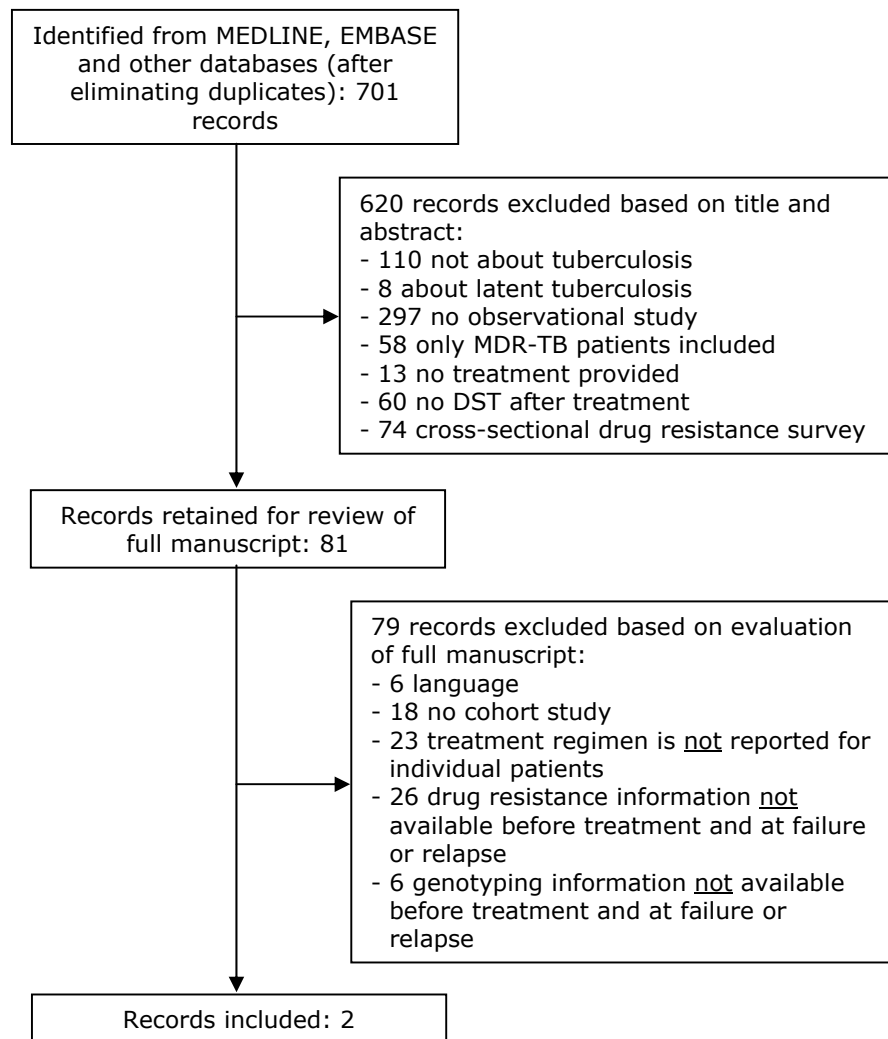
Figure 2: Forest and meta-analysis of the 2 included studies showing the risk ratio of inappropriate treatment and risk of developing multi-drug resistant TB.

Study or Subgroup	Inappropriate treatment Events	Inappropriate treatment Total	Appropriate treatment Events	Total	Weight	Risk Ratio IV, Fixed, 95% CI	Risk Ratio IV, Fixed, 95% CI
Cox 2007	9	74	1	240	66.4%	29.19 [3.76, 226.62]	
Matthys 2009	5	62	0	127	33.6%	22.35 [1.26, 397.86]	
Total (95% CI)		136		367	100.0%	26.68 [5.02, 141.70]	
Total events	14		1				

Heterogeneity: Chi² = 0.02, df = 1 (P = 0.88); I² = 0%
Test for overall effect: Z = 3.85 (P = 0.0001)

0.002   0.1   1   10   500
Favours Inappropriate use   Favours Appropriate use