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Prevalence and risk factors of multi-drug resistant tuberculosis in Dalian, China

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Abstract

Objectives: To investigate the prevalence and risk factors associated with multi-drug resistant tuberculosis (MDR–TB) in Dalian, China.

Methods: This was a retrospective review of data from patients attending a TB clinic in Dalian, China between 2012 and 2015. Demographic and drug susceptibility data were retrieved from TB treatment cards. Univariate logistic analysis was used to assess the association between risk factors and MDR–TB.

Results: Among the 3552 patients who were smear positive for *Mycobacterium tuberculosis* (MTB), 2918 (82.2%) had positive MTB cultures and 1106 (31.1%) had isolates that showed resistance to at least one drug. The overall prevalence of MDR–TB was 10.1% (359/3552; 131/2261 [5.8%] newly diagnosed and 228/1291 [17.7%] previously treated patients). Importantly, 75 extensively drug-resistant TB isolates were detected from 25 newly treated and 50 previously treated patients. In total, 215 (6.1%) patients were infected with a poly-resistant strain of MTB. Previously treated patients and older patients were more likely to develop MDR–TB.

Conclusions: The study showed a high prevalence of MDR–TB among the study population. History of previous TB treatment and older age were associated with MDR–TB.

Keywords

Tuberculosis, TB, multidrug resistant TB, drug resistant TB

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Introduction

Tuberculosis (TB) remains a global health problem.[1] Although many efforts have been made over the past 20 years to control TB, worldwide, there were an estimated 9 million new incident cases of TB in 2014.[1] The emergence of drug resistant TB over recent years has become a major threat in the control of the disease.[2] Two important types

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of drug resistant TB have been identified, multi-drug resistant (MDR)–TB and extensively drug-resistant (XDR)–TB. MDR–TB strains are resistant to isoniazid and rifampin, the standard, first-line drug treatments for TB; and XDR–TB stains are resistant to isoniazid and rifampin, at least one fluoroquinolone and any of the second-line injectable treatments (i.e. amikacin, capreomycin, kanamycin).[3] The World Health Organization (WHO) estimated that in 2014 there were 4.8 million new cases of MDR–TB worldwide and approximately 1.9 million associated deaths.[4] Moreover, compared with drug-susceptible TB, treatment for MDR–TB and XDR–TB can cost up to 25 times more and take three times longer to be effective.[5,6] Importantly, only approximately 20% of MDR–TB cases are correctly diagnosed and the strains are highly transmissible.[7]

With an estimated total number of 130 548 cases in 2014, China has the largest number of TB and MDR–TB cases in the world.[4] However, the drug resistance rate estimated by the WHO is inadequate for TB control for China.[1] Therefore, a study was conducted to determine the prevalence and characteristics of drug-resistant TB in a specific region in China with the aim of providing more accurate data on TB surveillance. Data from a TB centre in Dalian, a coastal city in the northeast of China with approximately 7 million inhabitants and a growing number of drug-resistant TB cases, were used to analyse drug-resistant TB and identify potential risk factors, especially for MDR–TB.

Patients and methods

Study design and patient population

This was a retrospective review of data from TB patients attending TB treatment clinics in Dalian, Liaoning Province, China between January 2012 and December 2015. Patient characteristics and details of drug susceptibility tests were retrieved from TB treatment cards and recorded by three researchers (X.T.L., X.Y.S., and X.W.L.). Newly treated patients were defined as those who had either never received anti-TB treatment or had received treatment for less than 1 month. Previously treated patients were defined as those who had received treatment for more than 1 month.[1] The retrospective nature of this study and that it used anonymized data meant that it did not require ethical committee approval or patient consent.

Microbiological methods

Two sputum samples had been collected from each patient with suspected TB. One of the samples, usually the first unless it was of poor quality, was used for culture. Smear microscopy and culture had been performed by a local laboratory while drug resistance testing had been conducted at a specialist centre. The Ziehl–Neelsen stain was used to confirm the presence of acid-fast bacilli in sputum smears. Positive smear samples were then cultured on Löwenstein–Jensen (LJ) culture medium with and without p-nitrobenzoic acid (PNB). Growth of *Mycobacterium tuberculosis* (MTB) is inhibited by PNB, whereas non-tuberculosis mycobacteria (NTM) are resistant. Incubation was at 37 °C for up to 8 weeks and the cultures were checked weekly for the growth of mycobacteria. Samples containing NTM were excluded from the study.

Drug susceptibility testing was performed on LJ medium impregnated with isoniazid (INH), rifampin (RIF), streptomycin (SM), ethambutol (EMB), ofloxacin (OFL) and kanamycin (KM) according to the proportion method as recommended by the WHO.[8] The concentrations of anti-TB drugs used were 0.2 mg/l for INH, 40 mg/l for RIF, 2 mg/l for EMB, 4 mg/l for SM, 2 mg/l for OFL, and 2 mg/l for KM. Any drug-resistant TB was defined as resistance to any of the anti-TB drugs among the six drugs recommend by the WHO (i.e. INH, RIF,

Table 1. Prevalence of anti-tuberculosis drug resistance in Dalian, China, 2012–2015.

Resistance profile	Newly treated patients $n = 2261$	Previously treated patients $n = 1291$	All smear positive cases $n = 3552$
Any TB drug resistance (total)	546 (24.1)	560 (43.4)	1106 (31.1)
INH	302 (13.4)	363 (28.1)	665 (18.7)
RIF	238 (10.5)	404 (31.3)	642 (18.1)
EMB	145 (6.4)	99 (7.7)	244 (6.9)
SM	121 (5.4)	96 (7.4)	217 (6.1)
OFL	161 (7.1)	219 (17.0)	380 (10.7)
KM	30 (1.3)	40 (3.1)	70 (2.0)
Mono-resistance (total)[a]	267 (11.8)	190 (14.7)	457 (12.9)
Poly-resistance (total)[b]	123 (5.4)	92 (7.1)	215 (6.1)
MDR–TB[c]	131 (5.8)	228 (17.7)	359 (10.1)
XDR–TB[d]	25 (1.1)	50 (3.9)	75 (2.1)

Data are expressed as *n* of patients (%).
[a]Mono-resistance: resistance to only one drug.
[b]Poly-resistance: resistance to two or more drugs excluding INH and RIF.
[c]MDR–TB: resistance to isoniazid and rifampin.
[d]XDR–TB: resistance to isoniazid and rifampin, at least one fluoroquinolone and any of the second line injectable treatments (e.g. kanamycin).
TB, tuberculosis; INH, isoniazid; RIF, rifampin; EMB, ethambutol; SM, streptomycin; OFL, ofloxacin; KM, kanamycin; MDR–TB, multi-drug resistant TB; XDR–TB, extensively drug-resistant TB.

EMB, SM, OFL or KM).[4] Mono-resistance was defined as resistance to only one drug and poly-resistance was defined as resistance to two or more drugs excluding INH and RIF.

Statistical analyses

Data analyses were performed using IBM SPSS software, version 19.0, for Windows® (IBM Corp, Armonk, NY, USA). Between group comparisons were made using χ^2-test and univariate logistic regression analysis was performed to assess the association between several risk factors and MDR–TB. A P-value < 0.05 was considered to indicate statistical significance.

Results

In total, 3552 smear positive patients were included in this retrospective study of whom, 2918 (82.2%) had positive MTB cultures. Of these 2918 patients, 1920 were new TB cases and 998 were previously treated patients. The sample was predominantly male (2232 men, 686 women) and the mean $\pm$ SD age was 50.79 ± 17.07 years.

Drug resistance status for all 3552 smear positive patients is shown in Table 1. Of the 3552 patients, 1106 (31.1%) had resistance to at least one of the six tested anti-TB drugs. Previously treated patients were significantly more likely to have drug resistance (43.4% [560/1291]) compared with newly treated patients (24.1% [546/2261]) ($P < 0.01$). The overall prevalence of MDR–TB was 10.1% (359/3552), of which 5.8% (131/2261) were newly treated patients and 17.7% (228/1291) were previously treated patients. Importantly, 75 (2.1%) patients had XDR–TB isolates of whom 25 (1.1%) were newly treated patients and 50 (3.9%) were previously treated patients. In addition, 215 (6.1%) patients had a poly-resistant strain of MTB; 5.4% (123/

Table 2. Comparison of age and sex of all smear positive patients with those who showed any drug resistance.

Variable	All smear positive isolates $n = 3552$	Any drug resistant isolates $n = 1106$	Statistical significance[a]
Age group, years			
0–30	574 (16.2)	152 (26.5)	
31–59	1930 (54.3)	657 (34.0)	
$\geq$60	1048 (29.5)	297 (28.3)	$P<0.001$
Sex			
Male	2680 (75.5)	840 (31.3)	
Female	872 (24.5)	266 (30.5)	NS

Data are expressed as *n* of patients (%); for 'Any drug resistant isolates', the percentage is calculated as a proportion of the corresponding 'All smear positive isolates' group and not of the total number of any drug resistant isolates.
[a]χ^2-test.
NS, no statistically significant between group difference ($P \geq 0.05$).

Table 3. Comparison of age and sex of all smear positive patients with those who showed multi-drug resistant tuberculosis (MDR–TB).

Variable	All smear positive isolates $n = 3552$	MDR–TB isolates $n = 359$	Statistical significance[a]
Age group, years			
0–30	574 (16.2)	53 (9.2)	
31–59	1930 (54.3)	220 (11.4)	
$\geq$60	1048 (29.5)	86 (8.2)	$P<0.05$
Sex			
Male	2680 (75.5)	277 (10.3)	
Female	872 (24.5)	82 (9.4)	NS

Data are expressed as *n* of patients (%); for 'MDR–TB isolates', the percentage is calculated as a proportion of the corresponding 'All smear positive isolates' group and not of the total number of MDR–TB isolates.
[a]χ^2-test.
MDR–TB defined as resistance to isoniazid and rifampin.
NS, no statistically significant between group difference ($P \geq 0.05$).

2261) of newly diagnosed patients and 7.1% (92/1291) of previously treated patients.

More than half the patients with smear positive TB (54.3%; 1930/3552) were in the 31–59-year age group. The age ranges for patients with any drug resistant isolates differed significantly ($P < 0.001$) compared with all smear positive patients (Table 2). While more men than women (75.5% versus 24.5%, respectively) had smear positive TB results, a similar proportion of men and women (31.3% versus 30.5%, respectively) had drug resistant isolates. However, the difference was not statistically significant (Table 2). Similar results were found for patients with MDR–TB; the age ranges differed significantly ($P < 0.05$) from all smear positive patients and the proportion of men and women with MDR–TB isolates was comparable (10.3% versus 9.4%, respectively) (Table 3).

Factors associated with the development of any drug resistance were investigated and previous anti-TB treatment was the most significant (odds ratio [OR] 3.20, 95% confidence interval [95% CI] 2.72, 3.76; $P < 0.001$) (Table 4). Being in the oldest age group (≥ 60 years) was the next most significant factor associated with any resistance (OR 1.30, 95% CI 1.09, 1.56; $P < 0.01$]. Sex was not associated with development of any drug resistance.

Factors associated with the development of MDR–TB were investigated and previous anti-TB treatment was the most significant (OR 4.44, 95% CI 3.57, 5.53; $P < 0.001$) (Table 5). Being in either of the older two age groups (31–59 and ≥ 60 years) was also a significant factor ($P < 0.05$). Sex was not associated with the development of MDR–TB.

The prevalence of drug resistant tuberculosis in Dalian, China over the years 2012–2015 is shown in Table 6. The prevalence of MDR–TB and XDR–TB decreased both in newly treated patients and previously treated patients from 2012 to 2015.

Table 4. Analysis of factors associated with any drug resistance.

	Any drug resistant isolates $n = 1106$	Odds ratio	95% confidence interval	Statistical significance[a]
Age group, years				
0–30	152/574 (26.5)			
31–59	657/1930 (34.0)	1.16	0.90, 1.50	NS
≥60	297/1048 (28.3)	1.30	1.09, 1.56	$P < 0.01$
Sex				
Male	840/2680 (31.3)			
Female	266/872 (30.5)	1.14	0.94, 1.37	NS
Previous treatment				
Yes	560/1291 (43.4)			-
No	546/2261 (24.1)	3.20	2.72, 3.76	$P < 0.001$

Data are expressed as *n* of patients (%) and the percentage is calculated as a proportion of the corresponding 'All smear positive isolates' group and not of the total number of any drug resistant isolates.
[a]Univariate logistic regression analysis.
NS, no statistically significant between group difference ($P \geq 0.05$).

Table 5. Analysis of factors associated with the development of multi-drug resistance tuberculosis (MDR–TB).

	MDR–TB $n = 359$	Odds ratio	95% confidence interval	Statistical significance[a]
Age group, years				
0–30	53/574 (9.2)			
31–59	220/1930 (11.4)	1.57	1.10, 2.25	$P < 0.05$
≥60	86/1048 (8.2)	1.48	1.15, 1.90	$P < 0.01$
Sex				
Male	277/2680 (10.3)			
Female	82/872 (9.4)	1.07	0.83, 1.38	NS
Previous treatment				
Yes	228/1291 (17.7)			
No	131/2261 (5.8)	4.44	3.57, 5.53	$P < 0.001$

Data are expressed as *n* of patients (%) and the percentage is calculated as a proportion of the corresponding 'All smear positive isolates' group and not of the total number of MDR–TB isolates.
[a]Univariate logistic regression analysis.
MDR–TB defined as resistance to isoniazid and rifampin.
NS, no statistically significant between group difference ($P \geq 0.05$).

Discussion

China has a high burden of TB and is a hot spot region for MDR–TB infection.[9] This current study presented the rates of drug resistance, including MDR–TB, collected from a TB Centre in Dalian, China over the period 2012 to 2015. The MDR–TB prevalence rates (overall 10.1%; newly treated patients 5.8% and previously treated patients 17.7%) were similar to those reported previously from ten provinces in

Table 6. Proportion of newly treated patients and previously treated patients with any drug resistant tuberculosis (TB), multi-drug resistant (MDR)–TB and extensively drug-resistant (XDR)–TB in Dalian, China, 2012–2015.

	Newly treated patients			Previously treated patients		
Resistance profile	*n*	Any TB resistance	MDR–TB[a] & XDR–TB[b]	*n*	Any TB resistance	MDR–TB[a] & XDR–TB[b]
Total	2261	546 (24.1)	156 (6.9)	1291	560 (43.4)	278 (21.5)
2012	160	63 (39.4)	26 (16.3)	358	206 (57.5)	106 (29.6)
2013	535	109 (20.4)	39 (7.3)	224	99 (44.2)	55 (24.6)
2014	793	211 (26.6)	52 (6.6)	374	175 (46.8)	79 (21.1)
2015	773	163 (21.1)	39 (5.0)	335	80 (23.9)	38 (11.3)

Data are expressed as *n* of patients (%).
[a]MDR–TB: resistance to isoniazid and rifampin.
[b]XDR–TB: resistance to isoniazid and rifampin, at least one fluoroquinolone and any of the second line injectable treatments (e.g. kanamycin).

China between 1996 and 2004 (overall 9.3%; newly treated patients 5.4% and previously treated patients 25.6%)[10] and from a national survey conducted in 2007 (overall 10.2%; newly treated patients 5.7% and previously treated patients 25.6%).[11]

In countries that have an effective TB control programme, the proportion of previously treated patients should be low.[12] However, the proportion of previously treated TB patients that had any TB resistance in this study was 43.4%. In addition, the results showed that previously treated patients were more likely to harbour drug-resistant TB and MDR–TB than newly treated patients. For example, the proportion of previously treated patients with MDR–TB was approximately four times greater than that of newly treated patients (17.7% versus 5.8%, respectively). The results of a univariate logistic regression analysis also showed that a history of previous treatment and older age were the most significant factors associated with MDR–TB. This finding is consistent with systematic reviews performed in Europe on risk factors associated with MDR-TB[13,14] and surveys conducted in several countries by the WHO.[4,9,15]

Importantly, the current investigation found 75 patients had XDR–TB. XDR–TB is associated with high morbidity and mortality and requires individualized treatment to address both first-line and second-line treatment resistance.[16,17] Surveillance data from China on XDR–TB are scarce.[18] However, China intends to expand an effective treatment programme for MDR–TB that is modelled on international best practice in an attempt to decrease the number of prevalent cases and reduce transmission of drug-resistant *M. tuberculosis*.[11,19] Although this current study observed high rates of MDR–TB and XDR–TB, particularly in previously treated patients, there was also a gradual reduction in prevalence over the years 2012–2015 in both newly and previously treated patients. Further studies on the changing drug resistant rates are required.

The study had several limitations. For example, risk factors for drug-resistant TB and MDR–TB were limited to age, sex and previous anti-TB treatment. In addition, these current data were only collected from Dalian and so may not reflect the situation from the whole Liaoning Province. Also, a proportion of culture negative samples for

MTB could have been due to extraneous factors such as the quality of the microscopic procedures, but overall this was not thought to have caused a systemic bias. However, further studies are required to determine the effect of other factors such as rural or urban location, education level, coverage provided by the 'directly observed treatment, short course' treatment strategy, smoking and concomitant diseases.

In conclusion, the rate of drug-resistant TB in Dalian was close to the nationally observed value.[11] Previously treated patients were more likely to develop drug-resistant TB as were older patients. Thus, the timely detection of drug resistance is of great importance in order to optimize patient treatment and to inform infection control measures designed to block the transmission of MDR–TB. Further work is required to understand the social and environmental determinants that contribute to high transmission rates. Moreover, a systematic study on the relationship between genotype and drug resistance is needed.

Declaration of conflicting interests

The authors declare that there are no conflicts of interest.

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