ANALYSIS AND SURVEILLANCE OF DRUG-RESISTANT *MYCOBACTERIUM TUBERCULOSIS* IN LIANYUNGANG COUNTY, CHINA

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Abstract. Drug-resistant tuberculosis (TB) has raised major global health concern. A lack of relevant information on frequency of *Mycobacterium tuberculosis* drug-resistant gene mutations has been a critical issue in China. The study determined epidemiology and drug resistance profiles of *M. tuberculosis* in Lianyungang County, China from 1 January 2014 to 31 December 2016 using specimens from the Tuberculosis Prevention and Control Agency, Lianyungang County from men (n = 1017) and women (n = 290), majority being 21-30 years of age. Overall resistance rate to four anti-TB drugs (isoniazid, kanamycin, ofloxacin, rifampicin) was 16.1%, with multidrug-resistant tuberculosis prevalence of initially treated patients of 19% and of retreated patients of 33%. Males and subjects in the younger (21-30 years of age) and older (62-70 years of age) age groups had the highest frequency of TB. Total drug resistance of *M. tuberculosis* showed a decreasing trend from 2014 to 2016. These findings can be used as reference baselines for future research on TB drug resistance.

Keywords: *Mycobacterium tuberculosis*, China, drug resistance, epidemiology, tuberculosis.

INTRODUCTION

Tuberculosis (TB) is reported to be a leading cause of death by a single infectious agent with an annual estimated 10.4 million new cases and 1.7 million mortality globally (WHO, 2017). The 5th National Epidemiological Survey of TB in China reported approximately 339,000 cases of multidrug-resistant (MDR-)TB and 105,000 cases with extensively drug-resistant (XDR-)TB in subjects >15-years of age. Isoniazid (INH) and rifampicin (RFP) are the two most commonly used first-line anti-TB drugs, widespread applications of which have caused the rise in drug resistance (Unissa *et al*, 2016, Campbell *et al*, 2001, Blanchard, 1996). Hence, control of incidences of MDR-TB cases is a top public health priority in China (Wang *et al*, 2020).

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The World Health Organization (WHO) established an ambitious End TB Strategy (WHO, 2015), with the aim of reducing 95% mortality and 90% incidence by 2035 using a 2015 statistics as a baseline. In order to reach this target, the Chinese government has been involved in extensive efforts to prevent and control MDR-TB (Ji *et al*, 2017, Pang *et al*, 2016). The international recommended short-course directly observed treatment (DOTS) strategy for treatment of active TB has currently proven to be a successful approach in TB control in endemic countries including China (Huynh *et al*, 2015).

Lianyungang County, a coastal region with a large population flux, is one of the first cities in China chosen for a pilot GeneChip project to detect *M. tuberculosis* (Wang *et al*, 2007). In conjunction with an increased use of DOTS, a 65% reduction in prevalence of smear-positive TB over the period 1990 - 2013 was reported from serial nationwide prevalence surveys (Zhu *et al*, 2017, Huynh *et al*, 2015, Wang *et al*, 2014).

This study was conducted in Lianyungang County using a drug susceptibility test to determine epidemiology and status of drug resistance of TB to provide reference baselines for developing more effective prevention and therapeutic strategies of *M. tuberculosis* infection.

MATERIALS AND METHODS

Clinical specimens

Sputum specimens were obtained from the Tuberculosis Prevention and Control Agency of Lianyungang County, China during 1 January 2014 to 31 December 2016. Sputum smear-positive specimens (n = 1,747) of suspected pulmonary TB patients were selected for evaluation by Löwenstein-Jensen (L-J) medium (Lianyungang CDC, Lianyungang, China) yielding 1,307 positive, 211 non-TB mycobacteria, 214 negative, and 15 contaminated cultures.

This study protocol was approved by the Medical Ethics Committee of the Fourth People's Hospital of Lianyungang (2019002). The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Prior written informed consent was waived because of the retrospective nature of the study.

M. tuberculosis culture

Culture samples were incubated in 4% NaOH with agitation and then cultured on slants of L-J medium at 37°C. Samples positive for acid-fast bacillus (AFB) following validation by Ziehl-Neelsen staining (https://www.cdc.gov/tb/default.htm) were selected for further study and negative results were confirmed if no bacteria growth appeared within 8 weeks. Sequenced *M. tuberculosis* H37RV from the Reference Laboratory of National Centre for Tuberculosis Control and Prevention, China CDC was used as reference strain.

Drug susceptibility testing (DST)

In brief, a 1.0 McFarland standard unit of mycobacterial suspension was prepared from a fresh culture and inoculated onto L-J slants with and without drugs (0.2 μg/ml INH, 30 μg/ml kanamycin (KM), 2 μg/ml ofloxacin (OFX), and 40 μg/ml RFP) and incubated at 37°C for 28 days. An isolate is considered resistant to a drug if there is ≥1% of colony growth on drug-containing medium compared to negative

control. MDR-TB strain is defined as a TB bacterial strain that is resistant to at least isoniazid and rifampicin, the two most potent TB drugs while XDR-TB strain is defined as a TB bacterial strain that is resistant to isoniazid and rifampicin, plus any fluoroquinolone (FQ) and at least one of three injectable second-line drugs (*ie*, amikacin, kanamycin, or capreomycin).

Statistical analysis

Information regarding the study subjects' age, gender, initial treatment and retreatment conditions, and drug susceptibility was primarily entered into Microsoft Excel 2010 and statistical analyses were subsequently performed using a Statistical Package for the Social Science (SPSS) software version 20.0 (Armonk, NY). A chi-squared test was used for statistical analysis and a p-value <0.05 based on a two-sided test is accepted as statistically significant.

RESULTS

Prevalence of *M. tuberculosis* in Lianyungang County

Of the 1,307 *M. tuberculosis* isolates collected in Lianyungang County from 1 January 2014 to 31 December 2016, 1,016 (77.8%) were from male patients and 291 (22.2%) from female, with a mean age of 51years (ranging from 1 year to 94 years), with highest prevalence among 21-30 years of age, followed by 61-70 years of age (Table 1). Among the five regions of Lianyungang County, highest prevalence (27.4%) was in the urban area and lowest (8.2%) in Guannan County (Fig 1).

Status of *M. tuberculosis* drug resistance

Over the period of study, there were 211 (16%) drug-resistant *M. tuberculosis* strains, with decreasing prevalence (χ^2 = 9.67, p-value = 0.008) (Table 2). Of the drug-resistant strains, the majority (33%) were resistant to INH, followed by MDR-TB (23%) but an equal number were resistant to OFX; prevalence of XDR-TB strains was low (2%) (Table 3). Prevalence of drug-resistant *M. tuberculosis* isolated from initially treated patients during the three study years, in general, is significantly lower than that from retreated patients (χ^2 = 25.53, p-value = 0.000), except for MDR-TB strains that was nearly 2 folds higher

Table 1
Age distribution of patients with tuberculosis in Lianyungang County, China (2014 to 2016).

Age group	Male (number)	Female (number)	Percent (n = 1,307)
0-10	1	1	0.2
11-20	39	18	4.4
21-30	178	78	19.6
31-40	64	29	7.1
41-50	117	36	11.7
51-60	175	39	16.4
61-70	207	43	19.1
71-80	152	29	13.8
81-90	81	17	7.5
91-100	3	0	0.2

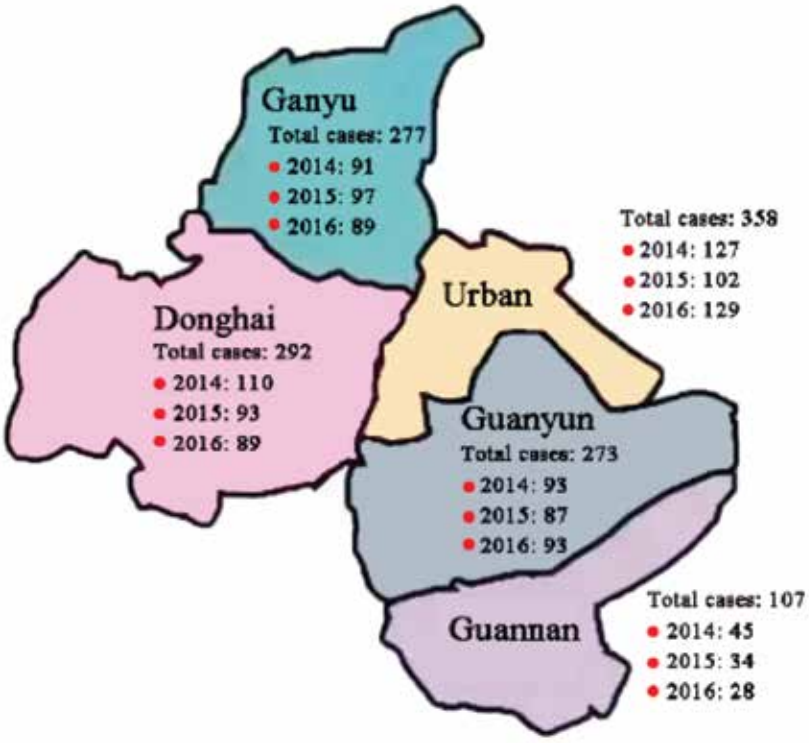

Fig 1-Distribution of patients with tuberculosis among the five regions of Lianyungang County, China (2014 -2016).

Table 2
Status of *Mycobacterium tuberculosis* strains to drugs obtained from patients in Lianyungang County, China (2014 to 2016).

Year	Number of drug* susceptible strains	Number of drug resistant strains	Total number of strains	Percent resistance
2014	371	95	466	20.0
2015	355	58	413	14.0
2016	370	58	428	13.0
Total	1,096	211	1,307	16.1

*Drugs used: isoniazid, kanamycin, ofloxacin, rifampicin.

in retreated patients ($\chi^2 = 4.215$, p-value = 0.04); this latter trend was observed with XDR-TB strains but the lower numbers precluded any statistical analysis. Over the three years of study, only prevalence of OFX resistance significantly decreased from 9% in 2014 to 5% in 2016 ($\chi^2 = 6.52$, p-value = 0.04).

Table 3
Drug-resistant *Mycobacterium tuberculosis* strains in initial-treated and retreated patients with tuberculosis in Lianyungang County, China (2014 - 2016).

Resistance	Initial treatment (n = 156)	Retreatment (n = 55)	Total (n = 211)
	Number of resistant strains (%)	Number of resistant strains (%)	Number of resistant strains (%)
To single drug			
RFP	18 (11)	7 (13)	25 (12)
INH	57 (36)	13 (24)	70 (33)
OFX	41 (26)	7 (13)	48 (23)
KM	3 (2)	0 (0)	3 (1)
To two drugs			
INH/OFX	3 (2)	3 (5)	6 (3)
INH/RFP	21 (13)	8 (14)	29 (14)
KM/OFX	2 (1)	0 (0)	2 (1)
KM/RFP	0 (0)	1 (2)	1 (0.5)
OFX/RFP	1 (0.5)	4 (7)	5 (2)
To three drugs			
INH/KM/OFX	1 (0.5)	2 (4)	3 (1)
INH/KM/RFP	1 (0.5)	0 (0)	1 (0.5)
INH/RFP/OFX	6 (4)	8 (14)	14 (7)
To four drugs			
INH/KM/RFP/OFX	2 (1)	2 (4)	4 (2)
MDR-TB	30 (19)	18 (33)	48 (23)
XDR-TB	2 (1)	2 (4)	4 (2)

INH: isoniazid; KM: kanamycin; MDR-TB: multidrug-resistant tuberculosis; OFX: ofloxacin; RFP: rifampicin; XDR-TB: extensively drug-resistant tuberculosis.

DISCUSSION

The occurrence of drug resistance in *M. tuberculosis* is of great concern globally because it threatens to roll back achievements made in halting and reversing TB (Bwalya *et al*, 2018). In most places of the world, including China, *M. tuberculosis* infection is higher in men than in women (Echazarreta *et al*, 2018, Dodd *et al*, 2016a, Oh *et al*, 2016). This phenomenon is probably associated with extrapulmonary TB, hospitalization, smoking, drug addiction and alcoholism among men (Echazarreta *et al*., 2018). In rural areas of Jiangsu, China, social activities and work stress are the main causes of high TB infection in men (Liu *et al*, 2017b).

The present study showed subjects of 21-30 and 61-70 years of age were the most susceptible groups to TB. The Global Tuberculosis Report, 2018 indicated high prevalence and mortality of TB among the elderly, with a peak among ≥65 years of age (WHO, 2018). In addition to poor compliance to medication and incomplete treatment in hospital, it is likely that

the majority of youths start their career with associated work burden and stress (Dodd *et al*, 2016b). In addition, living in a densely populated place, it is extremely important to prevent students from contracting TB infection at school and at home (Kawatsu *et al*, 2018).

Since DOTS strategy was implemented in Lianyungang County, management of drug-resistant TB patients has been well-established and a decline in prevalence of drug-resistant *M. tuberculosis* strains from 2014 to 2016 has been demonstrated making Lianyungang County's prevalence of drug-resistance TB lower than that of Jiangsu (16.92%) (Ding *et al*, 2017) and Yunnan (19.25%) (Bai *et al*, 2019). In addition, due to compliance to treatment and more qualified health service, prevalence of drug resistance in retreated group was lower than that of patients at initial treatment. The most common drug combination found in MDR-TB strains was INH/RFP, similar to that reported by (Liu *et al*, 2017a). It is worth noting Lianyungang County is one of the first cities in China employing GeneChip analysis to detect *M. tuberculosis* (Zhu *et al*, 2020) and the prevalence of MDR-TB strains in this county was lower compared to those of Changchun and Wuhan (Zhang *et al*, 2018, Huang *et al*, 2018).

According to WHO (2009), FQ is the most frequently used first-line treatment of drug-resistant TB, and KM, the second-line injection drug, enhances anti-TB chemotherapy effect with MDR-TB patients. OFX is also used to treat patients who are resistant to first-line anti-TB drugs (WHO, 2018). The higher prevalence of OFX resistance compared to that of KM may probably be due to the use of FQ to treat non-tuberculous respiratory infections.

In conclusion, outcome of drug resistance in *M. tuberculosis* with respect to age, gender, initial treatment, retreatment assessed among TB patients in Lianyungang County from 2014 to 2016 indicated protection of the young population (21-30 years of age) is a critical issue. Strategies should also be designed to reduce risk factors for TB in the male population. Public health intervention measures should focus on improving treatment and adherence to medication with particular attention paid to retreated group. The overall decline in frequency of anti-TB drug resistance demonstrated the success of DOTS-based programs in controlling spread of drug resistance. We will expand our samples to generalize to the rest of China and help with the construction of a prevention program for controlling TB spread. Extension of the present study to cover the rest of China should assist in the development of a prevention program for controlling TB. Policies that make preventative therapy more feasible on a large scale will help China achieve the global target of 95% reduction in mortality and 90% in incidence of TB by 2035.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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