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ORIGINAL ARTICLE

Genetic Profiling and Drug-resistance Pattern of Multidrug-resistant *Mycobacterium tuberculosis* Isolates from Patients and their Household contacts in North Indian Population

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Molecular characterization of *Mycobacterium tuberculosis* (MTB) is important to understand the pathogenesis, diagnosis, treatment, and prevention of tuberculosis (TB). However, there is limited information on molecular characteristics and drug-resistant patterns of MTB in patients with pulmonary tuberculosis (PTB) in India. Thus, this study aimed to determine the molecular characteristics and drug resistance patterns of MTB in patients with PTB in India.

Key words: Mycobacterium tuberculosis, prevention of tuberculosis, pulmonary tuberculosis, India

Tuberculosis (TB) remains a major global public health problem and is the leading cause of death from a single bacterium, *Mycobacterium tuberculosis* (MTB) complex. The emergence and spread of drug-resistant strains aggravate the problem, especially in tuberculosis high burden countries such as India. The supposedly high initial cost of laboratory diagnosis coupled with scarce financial resources has limited collection of information about drug resistance patterns and circulating strains in peripheral and emerging regions of India. Here, we investigated drug susceptibility and genetic diversity of mycobacterial isolates among pulmonary tuberculosis patients in the region and its surroundings in Eastern region of India.

The study of genetic diversity and drug susceptibility profile of *Mycobacterium tuberculosis* (MTB) will help understand the transmission dynamics and can be used for better prevention and control of the disease. The aim of this study was to determine the drug susceptibility profiles and genetic diversity using the random amplified polymorphic DNA (RAPD) and mycobacterial interspersed repetitive units-variable number of tandem repeats (MIRU VNTR) of MTB isolates from sputum samples of pulmonary TB patients residing in the two slums of Lucknow city in Uttar Pradesh, India.

Tuberculosis (TB), a highly contagious disease that sees no gender, age, or race is mainly a disease of lungs. According to World Health Organization, a TB patient can be completely cured with 6–9 months of anti-TB treatment under directly observed treatment short course.

Tuberculosis (TB) caused an estimated 1.3 million deaths worldwide in 2016 (WHO Global Tuberculosis Report, 2017). The major challenge in the treatment of tuberculosis is the emergence of drug-resistant *Mycobacterium tuberculosis* Gygli *et al.,* (2017). The drugs available for tuberculosis treatment are categorised into first-line (isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), ethambutol (EMB) and streptomycin (STR)) and second line (including fluoroquinolones, thioamides, cycloserine and the injectable aminoglycosides). Rising rates of multi-drug-resistant tuberculosis (MDR-TB, defined as resistance to INH and RIF), are of immense concern for TB control worldwide. Extended treatment is required with multiple drugs that have a higher rate of side effects but limited rate of treatment success (Gygli *et al*. 2017). India accounts for the highest burden of tuberculosis globally and also ranks top among the countries for MDR-TB cases (WHO Global Tuberculosis Report, 2017).

MATERIALS AND METHODS

Methodology Adopted:-

Patient Recruitment and Sample Collection: Sputum for acid-fast bacilli (AFB) smear microscopy and culture were collected from the index cases (IC) and household contacts (HC) during a contact investigation study on sputum smear-positive TB patients seen at the DOTS-PLUS Centre, Department of Respiratory Medicine King George's Medical University, Lucknow.

A total of Hundred Index patients, sputum smear-positive, with MDR-TB, were identified as per standard diagnostic criteria. Five hundred six (506) of their household contacts were evaluated for TB with tuberculin skin test (TST), chest x-ray and sputum examination.

Ninety two (18.1%) household contacts were identified positive by tuberculin skin test (TST).

Staining: Ziehl and Nelson method of staining were performed for all Samples.

Result: Out of 100 Index - 56 are 2+ or 3+ positive, 44 are 1+ or scanty.

A total of Seventy one (14%) household contacts with TB were identified positive by chest x-rays and/or positive sputum results; of these, 15 were sputum culture-positive.

Sample Processing: Sample processing was done within 24 hours by N-acetyl-L-cysteine (NALC) method.

Culture: Culture was done for 100 Index and 428 household contacts, sputum samples in liquid culture medium- Mycobacteria Growth Indicator Tubes (MGIT).

Result: A total of 98 Index Sputum samples and 15 household contacts shown positive culture growth in liquid culture medium.

DNA Isolation: DNA isolation done for 27 samples (12 index cases and 15 household contacts).

A total of 27 *M. tuberculosis* paired isolates were available from 12 index patients (IC) and 15 household contacts (HC)**.**

Collection of Patients Sample

Demographic and clinical data were gathered using data collection forms from the provincial laboratories on the basis of national TB codes, and the data were verified at the National TB Registry. The data collection was strictly anonymous and no patient identifiers were used throughout the collection and analysis period. The study has been reviewed and approved by the Office of Research Affairs at Department of Respiratory Medicine King George's Medical University, Lucknow.

RESULTS AND DISCUSSION

Drug susceptibility testing:

Only two of the 27 isolates were fully susceptible to all first line drugs; nine were resistant to all first line drugs.

Isoniazid (INH) resistance was detected in 25/27 and 19/21 at low (0.2 µg/ml) and high (1.0 µg/ml) concentrations, respectively.

Table 1. Demographic for Index case (IC): A detailed history with other social details of Index patients (100) is collected.

Variables	Factors	Frequency (n=100)	(%)
Sex	Male	67	67
	Female	33	33
Age	≤ 40	58	58
	>40	42	42
Residence	Urban	54	54
	Rural	46	46
Personal habit	Alcoholic	17	17
	Non-alcoholic	83	83
	Smoker	27	27
	Ex-smoker	4	4
	Non-smoker	69	69
Contact history	Present	7	7
	Past	9	9
	Absent	84	84
Smear Grading	3+ or 2+	54	54
	1+ or scanty	46	46
Culture Result	Positive	98	98
	Negative	2	2

Table 2. Demographic for household contact (HC): A detailed history with other social details of household contacts (506) is collected.

Variables	Factors	Frequency (n=506)	(%)
Sex	Male	342	67.6
	Female	164	32.4
Age	≤ 40	318	62.8
	>40	188	37.2
Residence	Urban	356	70.4
	Rural	150	29.6
Personal habit	Alcoholic	54	10.7
	Non alcoholic	401	89.3
	Smoker	94	18.5
	Ex-smoker	37	7.31
	Non-smoker	375	74.1
Smear Grading		(n=15)	
	3+ or 2+	11	73.3
	1+ or scanty	4	26.7
Culture Result		(n=428)	
	Positive	15	3.50
	Negative	413	96.5

Table 3. Drug susceptibilities of *Mycobacterium tuberculosis* isolates from index cases (IC) and household contact (HC) cases

Household/index contact	First line drugs				Second line drugs							
	INH		RIF	EMB	STR		CAP	KAN	AMI	ETH	CYC	PAS
	0.2	1.0	1.0	7.5	2.0	10	10	6.0	6.0	10	60.0	8.0
IC1	R	R	R	R	S	S	S	S	S	S	S	S
HC1	R	R	S	S	S	S	S	S	S	S	S	S
IC2	R	R	R	R	R	R	S	S	S	S	S	S
HC2	R	R	R	S	R	R	S	S	S	S	S	S
IC3	R	S	R	S	R	R	S	S	S	S	S	S
HC3.1	R	R	R	S	S	S	S	S	S	S	S	S
HC3.2	R	R	R	S	S	S	S	S	S	S	S	S
IC4	R	R	S	S	R	S	S	S	S	R	S	S
HC4	R	R	R	R	R	S	S	S	S	S	S	S
IC5	R	R	R	R	R	S	S	S	S	S	S	S
HC5	R	R	R	R	R	R	S	S	S	S	S	S
IC6	R	R	R	R	R	R	S	S	S	R	S	S
HC6.1	R	R	R	R	R	R	S	S	S	R	S	S
HC6.2	S	S	S	S	S	S	S	S	S	S	S	S
IC7	R	S	R	R	R	R	S	S	S	R	S	S
HC7	R	S	R	R	R	R	S	S	S	S	S	S
IC8	R	S	S	S	S	S	S	S	S	S	S	S
HC8.1	S	S	S	S	S	S	S	S	S	S	S	S
HC8.2	R	R	R	S	S	S	S	S	S	S	S	S
IC9	R	R	R	R	R	S	S	S	S	S	S	S
HC9	R	R	R	R	S	S	S	S	S	S	S	S
IC10	R	S	S	S	S	S	S	S	S	S	S	S
HC10	R	S	S	S	S	S	S	S	S	S	S	S
IC11	R	R	S	S	S	S	S	S	S	S	S	S
HC11	R	R	R	S	S	S	S	S	S	S	S	S
IC12	R	R	R	R	R	R	R	S	S	S	S	S
HC12	R	R	R	R	R	R	R	S	S	S	S	S

Table 4. Patient demographic

Index Patient/House hold contacts	Year diagnosed with tuberculosis	Years between diagnosis	Age of diagnosis	Sex	Relationship	DST matches? (Y/N)
IC1	2015		41	F		
HC1	2015	0	22	F	Child	No
IC2	2015		28	M		
HC2	2015	0	18	M	Sibling	No
IC3	2015		52	M		
HC3.1	2015	0	22	F	Child	No
HC3.2	2015	0	17	M	Sibling	No
IC4	2016		38	M		
HC4	2016	0	10	M	Child	No
IC5	2016		42	M		
HC5	2016	0	22	M	Child	Yes
IC6	2016		17	M		
HC6.1	2016	0	20	M	Sibling	Yes
HC6.2	2016	0	29	M	Sibling	No
IC7	2016		43	M		
HC7	2017	1	33	F	Sibling	Yes
IC8	2016		28	F		
HC8.1	2017	.3	60	M	Parent	No
HC8.2	2017	NA	41	M	Sibling	No
IC9	2017		19	M		
HC9	2017	0	30	M	Sibling	Yes
IC10	2017		40	M		
HC10	2017	0	40	F	Sibling	Yes
IC11	2017		41	M		
HC11	2017	0	12	M	Child	No
IC12	2017		30	F		
HC12	2017	0	28	F	Sibling	No

Resistance to rifampin (RIF) and ethambutol (EMB) occurred in 19/27, 14/27, respectively.

With second line drugs, resistance to streptomycin (STR) at 2.0 µg/ml, streptomycin at 10 µg/ml, and ethionamide (ETH) was detected in 14/27, 10/27, 5/27 respectively.

Note:

Susceptibilities are reported in µg/ml.

Abbreviations: INH – isoniazid, RIF – rifampin, EMB – ethambutol, PZA – pyrazinamide, STR – streptomycin, CAP – capreomycin, KAN – kanamycin, AMI – amikacin, ETH – ethionamide, CYC – cycloserine, PAS – para-aminosalicylic acid; R – resistant, S – susceptible.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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