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Phenotypic Detection of Rifampicin and Isoniazid Resistance Pattern of *Mycobacterium Tuberculosis* Auramine Positive Isolates in Mtata, South Africa

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ABSTRACT

Background: *The increase prevalence of Isoniazid (INH) and Rifampicin (RIF) resistant Tuberculosis (TB) has become a significant challenge for TB control for over 10 years.*

Aim: *The study assessed the phenotypic detection of rifampicin (RIF) and isoniazid (INH) resistant pattern of Mycobacteria tuberculosis among patients attending hospitals in Mtata, South Africa.*

Methodology: *Three hundred and fifty (350) patients age between 10-80 years attending Nelson Mandela Hospital Mtata were recruited for the study. Ethical approval was obtained from the Ethics Committee of Nelson Mandela Hospital Mtata. Sputum specimens were investigated employing Auramin fluorescence microscopy technique. Drug susceptibility testing (DST) was carried out using cultural technique and conventional drugs susceptibility testing method.*

Results: *Result showed that 28.6% of the isolates were positive on culture. For INH testing, 27% were sensitive, 68% were resistant where as 5% had lost viability to INH. For RIF, 28% were sensitive, 69% were resistant and 3% had lost viability to RIF. The rural settlers has 55% INH resistance and 26% RIF resistance while in urban area 35% and 25% were resistance to RIF and INH respectively.*

Conclusion: *Result from this study showed that INH and RIF resistance were very high among the patients attending Nelson Mandela Hospital and other treatment centers in Mtata, South Africa.*

Keywords

Drug sensitivity testing (DST), Micobacterium tuberculosis (MBT), Auramin, Isoniazid (INH), Rifampicin (RIF).

Introduction

Mycobacterium tuberculosis (MBT) infection remains a common infectious disease worldwide, with up to 10 million reported cases and 2 million deaths per year [1]. Meanwhile, the incidence of this disease seemed to have declined during the 1970s and early 1980s because of the success in chemotherapy. However, there has been a global resurgence of tuberculosis since the late 1980s [2]. In Hong Kong, 6607 cases were recorded in the year 2002, at the rate of 97/100 000 people [3]. Tuberculosis (TB) has been reported from all parts of the world, however, over 95% of cases and deaths occur in developing countries [4].

Multidrug Resistance – Tuberculosis (MDR- TB) rate of 2.6% among new cases and 17.6% among retreatment cases was reported at the latest National Survey in Nepal [5]. The emergence of MDR-TB is widely considered to be a serious threat to global TB control, and in Nepal, being geographically situated between China and India which carry almost 50% of worlds MDR- TB burden, drug resistance TB is emerging as a national problem [6-8]. Isoniazid (INH) is one of the most effective agents for the treatments of tuberculosis and is the backbone of the modern short-course. As one of the first-line anti –TB drugs, INH has been used in TB

control programs for greater than 50 years and for the treatment of latent Mycobacterium tuberculosis (LMTB) Infection to prevent the active disease and subsequent TB transmission. However, the rapid increase of INH resistance in recent years poses a serious threat to global TB control, especially in China. WHO report 2008, estimated that the INH resistance rate in China is 41.2%, which is much higher than the global estimates of 13.3% [9]. Considering the 13.1% estimated INH resistance rate in 2000 reported by National survey [10]. The dissemination of INH resistance TB in china is dramatically high in the last 10 years. The speedy dissemination of INH resistance is of public health concern as it could lead to increased prevalence multidrug resistant TB (MDR-TB). MDR-TB is defined as TB resistance to at least INH and RIF in some instances to as many as seven anti-TB drugs [11]. By now, China has the largest number of MDR- TB cases in the world, and many extensively drug- resistant TB cases have been reported [12,13]. Recent increases both in INH- resistant and multi drug –resistant (MDR) tuberculosis have been jeopardizing the effects of global TB control through the implementation of the directly observed treatment short course (DOTS) [14,15].

In China, DOTS program was adopted since early 1990s, but the increased prevalence of drug resistant TB has become a significant challenge for TB control in last ten years. The prevalence of MDR-TB and INH resistant was estimated at 8.3% and 41.2 % respectively among all cases in china which were both high compared to the global estimates of 4.8% and 13.3% [16]. The development of INH resistance is a common first step in the evolution to MDR [17]. Thus, there has been considerable interest in identifying the molecular basis of INH resistance and understanding the transmission pattern of the INH resistant MTB strain.

Materials and Method

This study was conducted in TB department Nelson Mandela Hospital, Mtata, South Africa. A 350-sputum specimen were obtained from patients on DOTS and patients on treatment among rural and urban settlers.

Methods

Specimen were obtained in a sterile, leak proof, wide mouth transparent and stoppered plastic containers. Sputum smears were made on slides, stained using Auramine, and subsequently viewed using fluorescence microscopy. All acid fast positive specimen were cultured on Lowenstein –Jensen (LJ) medium. Culture positive specimen were assessed for drug resistance using phenotypic conventional DST. Proportion method was used on LJ medium for DST at critical concentration of 0.2 ug/ml for INH and 40 ug/ ml for RIF. The bacterial culture plates were read after 6 weeks of incubation at 37°C and the strains were considered resistant if the proportion of resistant bacteria was higher than 1%.

Statistical Methods

Chi square was used for analysis of values. Level of significance was set at $p < 0.05$. The statistical analysis was done using SPSS statistical software package (version 21).

Results

Table 1: Total Sample Treated With %AUR Positive.

Total sample	AUR +ve	Total % AUR +ve
350	100	28. 6

Table 2: Various Ages of the Patients Sampled.

Age	Samples	AUR +ve	% AUR +ve	AUR -ve
10-20	50	5	5	45
21-30	50	10	10	40
31-40	50	13	13	37
41-50	50	15	15	35
51-60	50	15	15	35
61-70	50	20	20	30
71-80	50	22	22	28
Total	350	100		

Table 3: Rate of Positive MBT among Rural and Urban Settler.

Location	Sample from location	AUR +ve	% AUR +ve	AUR -ve
Rural Settlers Ages 10-80	175	70	70	105
Urban Settlers Ages 10-80	175	30	30	145
	350	100	100	

Table 4: Indicates Percentage Resistance and Sensitive of INH Respectively.

Ages	INH	INH S	INH R	% INH R	% INH S	DOT	LV
10-20	5	4	1			+	-
21-30	10	5	4			+	1
31-40	13	2	9	68	27	-	2
41-50	15	2	13			-	-
51-60	15	2	12			-	1
61-70	20	4	16			-	-
71-80	22	8	13			+	1
	100	27	68				

Table 5: Percentage Resistance and Sensitive of RIF Respectively.

Ages	RIF	RIF S	RIF R	% RIF R	% RIF S	DOT	LV
10-20	5	3	2			+	-
21-30	10	4	6			+	-
31-40	13	5	8	69	28	-	-
41-50	15	4	11			-	
51-60	15	3	10			-	2
61-70	20	4	16			-	-
71-80	22	5	16				1
	100	28	69				

Table 6: Percentage Resistance of INH among Rural Settlers.

Location	Sample	Aur +ve	% Aur	INHR	INHS	% INHR	% INHS	LV	DOT
Rural									
Settlers	175	70	70	35	30	35	30	5	-
Ages									
10-80									

Table 7: Percentage Resistance of RIF among Rural Settlers.

Location	Sample	Aur +ve	% Aur	RIFR	RIFS	% RIFR	% RIFS	LV	DOT
Rural									
Settlers	175	30	30	26	4	26	4	-	-
Ages									
10-80									

Table 8: Percentage Resistance of RIF among Urban Settlers.

Location	Sample	Aur +ve	% Aur	RIFR	RIFS	% RIFR	%RIFS	LV	DOT
Urban									
Settlers	175	55	55	24	27	24	27	4	+
Ages									
10-80									

Table 9: Percentage Resistance of INH among Urban Settlers.

Location	Sample	Aur +ve	% Aur	INHR	INHS	% INHR	% INHS	LV	DOT
Urban									
Settlers	175	45	45	25	18	25	18	2	+
Ages									
10-80									

List of Abbreviations:
MTB: Mycobacterium Tuberculosis; INHR: Isoniazid Resistant; RIF: Rifampicin; INH: Isoniazid; RIFR= Rifampicin Resistant; MDR: Multidrug – Resistant; AUR: Auramin; DOTS: Directly Observed Treatment Short Course; LV: Lost Viability; DST: Drug Susceptibility testing.

Discussion

Tuberculosis, one of the oldest recorded human diseases is still one of the biggest killers among the infectious diseases ravaging the globe despite the use of a live attenuated vaccine and several antibiotics. The development of drug resistance in the population has increased the possibility that Tuberculosis may once again become an incurable disease [18]. The emergence and spread of DR- TB threatens TB control and has become a major Public Health problem in South Africa. Development of DR- TB, including MDR, is attributed to poor patients' compliance, inappropriate TB drug regimen, inadequate laboratory facilities for drug susceptibility testing, and acceleration of human immunodeficiency virus epidemic [19-22]. Resistance to drugs is due to particular genomic mutations in specific genes of MTB. To date, nine genes are known to be linked to resistance to first – line anti-TB drug; katG, inhA, aphC, and kasA for INH resistance, rpoB for RIF resistance , rpsl and rrs for STR resistance, embB for EMB resistance, and pncA for PZA resistance, resistance to multiple drugs is the consequence of an accumulation of mutation [23]. TB is a serious public health problem all over the world and a most common and fatal infectious disease in china accounting for 43% of deaths from infectious diseases [24]. This issue has become more complicated because of the emergence of multiple drug resistance in the last decade and lack of newly developed new anti-TB drugs. Resistance to INH a first line anti-TB drug is the foundation of multidrug resistance. Out of 350 specimen that were investigated, 28.6% (100) were auramin positive of which 68% (68) were INH resistant were as 69% (69) were RIF resistant. The finding in (table 5) revealed that RIF had the highest resistance (69%) followed by INH (68%). This is difference from the findings of Annelies et al. [25] reported that resistance was 93%, 56%, 41%, and 18% to INH, RIF, STR and EMB respectively and a lower prevalence of resistance was reported by Rivera et al. [26] who studied 188 MTB isolate in Philippines and noted that drug resistance represented 17, 6% of isolates and in Qatar, by Al – Marri [27] who found 6 (15%) among 406 MTB isolate. It was discovered that rural settlers are more affected with TB compare to urban settlers with a higher multidrug resistance. The rural settlers has 55% INH resistance and 26% RIF resistance while in urban area there is less percentage of 35% and 25% of RIF and INH respectively. This may be non-implementation of directly observed therapy short course (DOTS) among the rural settlers. In my study, the incidence of drug resistant is higher compared to that reported in a previous study conducted by Massi et al. [28] in Makassar, Indonesia, where the proportion of Mycobacterium tuberculosis resistant to at least one first – line TB drug reached 40% in new and retreatment cases. The proportion of DR in my study is also higher than that reported Nana et al. [29] in China where 33.3% isolate were resistant to all first line TB drugs. The finding in tables 6 and 7 of the rural settlers have higher percentage of INHR and RIFR with 35% and 26% respectively compared to table 8 and 9 of the urban settlers with percentage of RIFR and INHR 24% and 25% respectively , this is attributed to the rural settlers not observing or making use of DOT. It was discovered that rural settlers are more affected with TB compare to urban settlers with a higher multidrug resistance.

Conclusion

Result from this study showed that INH and RIF resistance were very high among the patients attending Nelson Mandela Hospital and other treatment centers in Mtata also rural settlers are more affected with a high multidrug resistance than urban settlers in South Africa.

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