

First Line Anti-Tuberculosis Drug Resistance Pattern in *Mycobacterium tuberculosis* Isolates at the University of Santo Tomas Hospital from 2003-2013

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Abstract

Introduction: Development of drug resistance is one of the most important barriers in achieving global control of tuberculosis (TB). Continuous surveillance, such as observation of susceptibility and resistance patterns to anti-TB drugs, together with nationwide programs aimed at TB case identification, treatment and control, physician and patient education, is a valuable tool in the goal towards reducing TB prevalence and mortality.

Objective: It is the aim of this study to determine the prevalence rate and resistance pattern of first line anti-tuberculosis drugs in a tertiary hospital in Manila, Philippines

Materials and Methods: Records of specimens submitted for *Mycobacterium tuberculosis* (MTB) culture and sensitivity, using BACTEC™ MGIT™ 960 SIRE Kit and PZA Kit, at the Section of Clinical Pathology, University of Santo Tomas Hospital, were reviewed. Isolates cultured for MTB were subjected to sensitivity studies to rifampicin (R), isoniazid (H),

ethambutol (E), pyrazinamide (Z) and streptomycin (S).

Results: A total of 546 specimens were cultured for MTB and sent for sensitivity studies. Majority of the specimens were from pulmonary sources (77%). Overall resistance rate was 52.38% (n=286). One-drug resistance was 23.26% (n= 127; highest with R followed by H); two-drug resistance was 15.38% (n=84; highest with H-R); three-drug resistance was 8.61% (n=47; highest with H-R-E and H-R-S); four-drug resistance was 4.58% (n=25; highest with H-R-E-S) and five-drug resistance (H-R-E-S-Z) rate was 0.55% (n=3).

Conclusion: The University of Santo Tomas Hospital, as a referral facility, is encountering an increasing number of drug-resistant tuberculosis from 2003 to 2013.

Keywords: Anti-tuberculosis drugs; Drug Resistance; *Mycobacterium tuberculosis*; University of Santo Tomas Hospital

Introduction

According to the World Health Organization (WHO) Global Tuberculosis Report 2014¹, there are 9 million new cases of tuberculosis (TB) this year, with 1.5 million dying due to this disease. The Philippines ranks 8th in incidence and seventh in prevalence among 22 high-burden countries, with an estimated mortality of 27,000 in the last year.¹ It also ranks seventh among 27 high-burden countries for Multi-Drug Resistant TB (MDR-TB).¹

In the recent National Drug Resistance Survey on TB², the estimated proportion of MDR-TB for new and retreatment cases has been reported at 2% and 21%, respectively; while resistance to any first-

line drug is at 17.47% for new cases and 43.61% for previously treated cases. In a 2012 study by the Tropical Disease Foundation³, among 4,897 patients screened for MDR-TB from 2003-2008, the overall drug resistance rate was noted at 81%. In Cebu and Mandaue cities, city and hospital-based reports on MDR-TB⁴ showed an overall drug resistance rate of 27.8%, with 3.9% among new cases and 46.4% in those previously treated.⁷ Drug resistance surveillance at the University of Santo Tomas Hospital (USTH) showed significant decline from 71.3% in 1990-1994 to 51.35% in 1995-2000⁵, but slightly increased again to 52.38% in 2003-2007⁶. The same pattern was observed with isoniazid resistance, declining from 34% to 24.7%, and increasing to 28.97%. Resistance to rifampicin was erratic during the same period, declining from 28% to 19%, then increasing to 30.92% in the last study.

MDR-TB is primarily a man-made phenomenon brought about by inappropriate, inadequate or poorly compliant treatment.⁷ This is a problem, particularly in low-income countries where culture and Drug Susceptibility Testing (DST) are not readily available. Globally, only 8.5% of new bacteriologically-confirmed

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TB cases and 17% of patients previously treated for TB were tested for MDR-TB in 2013. Locally, the DST coverage is only at 3.2% and 70% respectively, and this resulted in gross underestimation of MDR-TB cases.¹

Continuous surveillance of susceptibility and resistance patterns to anti-TB drugs is a valuable tool in guiding clinicians in treating TB when available and accessible. There is a need to analyze the trends of resistance over the years to address the therapeutic requirements of patients.

In view of the aforementioned, the objectives of this study are: 1) to determine the prevalence rate of drug-resistant TB in specimens submitted at the USTH Laboratory from 2003-2013 and the frequency of positive isolates among the various types of specimens; 2) to determine the resistance pattern of anti-TB drugs, and compare them with available data from prior studies.

Methodology

Patients, of any age and gender, with specimens (pulmonary and extrapulmonary) submitted for TB culture and sensitivity at the USTH Laboratory from 2008-2013 were included in the study. Specimens yielding *Mycobacterium* other than tuberculosis (MOTT) and MTB Complex with no sensitivity studies were excluded. Incomplete data were not included as well.

Records of patients at the USTH Laboratory from January 2008 to December 2013, with specimens (respiratory system — sputum, bronchial aspirates, endotracheal secretions, pleural fluid, pleura and lung tissues; and extrapulmonary sources — fluids, secretions, tissues from other organs e.g., synovial fluid, cerebrospinal fluid) submitted for TB culture and sensitivity, using BACTEC™ MGIT™ 960 SIRE Kit and PZA Kit, were reviewed (Appendix A). A letter of request for permission was likewise sent and approved by the medical director and head of the USTH Department of Clinical Pathology to access these results. Institutional Review Board approval was also sought prior to the commencement of this study. The data gathered from the laboratory logbook included the specimen number, date of examination, type of specimen, culture result and drug susceptibility to isoniazid (H), rifampicin (R), ethambutol (E), streptomycin (S) and pyrazinamide (Z). Susceptibility was labeled as Susceptible or Resistant. The total number of isolates was counted and classified as to which ones were sensitive and resistant to the assigned drugs. The results were then combined and compared with data gathered from 2003-2007⁶, and the national data based on the WHO Global TB Report 2014¹ and Philippine Drug Resistance Surveillance 2012-2013².

Results

MTB Positive Cultures

A total of 4,881 specimens were submitted within the study period. Five hundred forty six cultures of MTB were isolated from 2003-2013 (n=359 from 2003-2007⁶, n=187 from 2008-2013), and sent for sensitivity testing (Table I). The 339 specimens with culture results but no sensitivity testing were excluded. Majority of the specimens were from pulmonary sources (77%), most common of which was from sputum. Non-pulmonary sources composed of 23%, majority of which was from urine specimen. (Table II)

Table I: Specimens that yielded positive for MTB Culture and sent for sensitivity testing at the University of Santo Tomas Hospital 2003-2007⁶, 2008-2013

Year	Total (%)
2003	58 (10.62%)
2004	119 (21.79%)
2005	74 (13.55%)
2006	53 (9.70%)
2007	55 (10.07%)
2008	26 (4.76%)
2009	43 (7.88%)
2010	41 (7.51%)
2011	18 (3.30%)
2012	30 (5.49%)
2013	29 (5.31%)
TOTAL	546

MTB Positive Cultures Susceptibility and Resistance Rates in 2008-2013

Sensitivity rate to the five drugs (H-R-E-Z-S) was 47.62% (85/187). The resistance to one drug alone was computed at 22.46% (42/187) with resistance to isoniazid being the highest at 6.95% (42/187). Two-drug resistance was at 14.44% (27/187) with H-R combination as the highest at 8.56% (16/187). Three-drug resistance was at 9.09% (17/187) with H-R-Z combination at 2.67% (5/187). Four-drug resistance was at 7.49%, the highest of which is the H-R-S-Z at 4.28%. Pan-resistance is at 1.07%. (Table III)

Overall MTB Positive Cultures Susceptibility and Resistance Rates in 2003-2013

When combined with the data from 2003-2007⁶ (Table IV), overall resistance rate was 52.38% (n=286). One-drug resistance was 23.26% (n= 127; highest with R followed by H); two-drug resistance was 15.38% (n=84; highest with H-R); three-drug resistance was 8.61% (n=47; highest with H-R-E and H-R-S); four-drug resistance was 4.58% (n=25; highest with H-R-E-S) and five-drug resistance (H-R-E-S-Z) rate was 0.55% (n=3). (Table V)

Table II: Isolates obtained for MTB culture and sensitivity from 2003-2013

	2003-2007 ⁶	2008-2013	TOTAL
PULMONARY	289 (80.50%)	134 (71.66%)	423 (77%)
Bronchial Aspirate	2 (5.57%)	2 (1.07%)	4 (0.7%)
Bronchial Washing	7 (1.95%)	7 (3.74%)	14 (2.56%)
Endotracheal Tube Aspirate	6 (1.67%)	2 (1.06%)	8 (1.47%)
Lung	7 (1.95%)	3 (1.60%)	10 (1.83%)
Pleural Fluid	12 (3.34%)	13 (9.70%)	25 (4.58%)
Pleural Tissue	2 (0.56%)	4 (2.14%)	6 (1.10%)
Sputum	253 (70.47%)	103 (55.08%)	356 (65.20%)
NON-PULMONARY	70 (19.50%)	53 (28.34%)	123 (23%)
Abdominal Abscess	0 (%)	1 (0.53%)	1 (0.18%)
Abscess	1 (0.28%)	4 (2.14%)	5 (0.92%)
Wound Discharge	3 (0.84%)	3 (1.60%)	6 (1.10%)
Bone Tissue/Discharge	1 (0.28%)	4 (2.14%)	5 (0.92%)
Ileocecal Mass	4 (1.11%)	3 (1.60%)	7 (1.28%)
Lymph Node	0 (0%)	2 (1.07%)	2 (0.37%)
Cerebrospinal Fluid	5 (1.39%)	11 ((5.88%)	16 (2.93%)
Joint Tissue/Discharge	1 (0.28%)	4 (2.14%)	5 (0.92%)
Liver	1 (0.28%)	3 (1.60%)	4 (0.73%)
Mediastinal Mass	0 (0%)	1 (0.53%)	1 (0.18%)
Neck Mass/Discharge	1 (0.28%)	3 (1.60%)	4 (0.73%)
Tissue	4 (1.11%)	2 (1.07%)	6 (1.10%)
Paravertebral Mass	1 (0.28%)	1 (0.53%)	2 (0.37%)
Peritoneal Fluid	1 (0.28%)	1 (0.53%)	2 (0.37%)
Scrotal Abscess	0 (0%)	1 (0.53%)	1 (0.18%)
Chest Wall Tissue	0 (0%)	1 (0.53%)	1 (0.18%)
Umbilical Hernia	0 (0%)	1 (0.53%)	1 (0.18%)
Urine	29 (8.07%)	1 (0.53%)	30 (5.49%)
Psoas Muscle Discharge	1 (0.28%)	1 (0.53%)	2 (0.37%)
Spinal Mass	0 (%)	3 (1.60%)	3 (0.55%)
Gastric Aspirate	2 (0.58%)	0 (0%)	2 (0.37%)
Colonic Ulcer	1 (0.28%)	0 (0%)	1 (0.18%)
Bladder Tissue/Washing	2 (0.58%)	0 (0%)	2 (0.37%)
JP Drain	1 (0.28%)	0 (0%)	1 (0.18%)
Intrahepatic Duct	1 (0.28%)	0 (0%)	1 (0.18%)
Retropharyngeal Mass	1 (0.28%)	0 (0%)	1 (0.18%)
Pericardial Fluid	1 (0.28%)	0 (0%)	1 (0.18%)
Inguinal Discharge	1 (0.28%)	0 (0%)	1 (0.18%)
Synovial Fluid/Tissue	3 (0.84%)	1 (0.53%)	4 (0.73%)
Bile	3 (0.84%)	0 (0%)	3 (0.55%)
Vitreous Fluid	1 (0.28%)	0 (0%)	1 (0.18%)

Individual First Line Drug Resistance Rate in 2003-2007⁶, 2008-2013

The overall individual drug resistance rate is still highest with isoniazid (32.23%) and rifampicin (29.30%). There was a noted slight increase in drug resistance

Table III: MTB positive cultures susceptibility and resistance rates from 2008-2013

	2008	2009	2010	2011	2012	2013	TOTAL	%
None	7	20	25	7	15	11	85	47.62
H	1	6	2	1	0	3	13	6.95
R	4	0	2	1	2	0	9	4.81
E	0	1	1	0	0	0	2	1.07
S	2	2	1	1	1	0	7	3.74
Z	0	2	0	2	3	4	11	5.88
H-R	4	3	4	4	1	0	16	8.56
H-E	1	0	0	0	0	0	1	0.53
H-S	3	0	2	0	0	0	5	2.67
R-E	0	0	0	0	0	0	0	0
R-S	0	0	0	0	0	0	0	0
E-S	0	0	0	0	0	0	0	0
H-Z	0	0	0	0	5	0	5	2.67
R-Z	0	0	0	0	0	0	0	0
E-Z	0	0	0	0	0	0	0	0
S-Z	0	0	0	0	0	0	0	0
H-R-E	0	1	0	0	0	2	3	1.6
H-R-S	2	1	0	0	0	1	4	2.14
R-E-S	0	0	0	0	0	0	0	0
H-E-S	0	0	0	0	0	1	1	0.53
H-R-Z	0	2	0	0	1	2	5	2.67
H-E-Z	0	1	0	0	0	0	1	0.53
H-S-Z	0	1	1	0	0	1	3	1.6
R-E-Z	0	0	0	0	0	0	0	0
R-S-Z	0	0	0	0	0	0	0	0
E-S-Z	0	0	0	0	0	0	0	0
H-R-E-S	2	1	0	1	0	0	4	2.14
H-R-E-Z	0	0	0	0	1	1	2	1.07
H-R-S-Z	0	2	3	1	1	1	8	4.28
H-E-S-Z	0	0	0	0	0	0	0	0
R-E-S-Z	0	0	0	0	0	0	0	0
H-R-E-S-Z	0	0	0	0	0	2	2	1.07

H=Isoniazid R=Rifampicin E=Ethambutol S=Streptomycin Z=Pyrazinamide

with isoniazid and streptomycin in 2008-2013, compared with 2003-2007, while a decreasing trend of resistance rate was seen with rifampicin and ethambutol. There seemed to be a dramatic increase in pyrazinamide resistance from 2003-2007 to 2008-2013. This may be because the pyrazinamide testing kit was only made available in our institution in August 2007, and was not able to record resistance in the previous years. (Table VI)

There was an increasing trend of drug susceptibility in 2003-2007 from 20.69% in 2003 to 52.73% in 2007, with the highest susceptibility noted in 2004 at 60.50%.

Table IV: MTB positive cultures susceptibility and resistance rates from 2003-2007^a

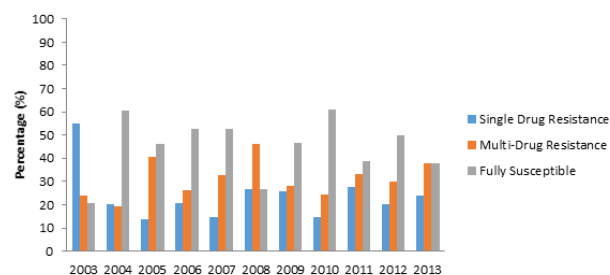
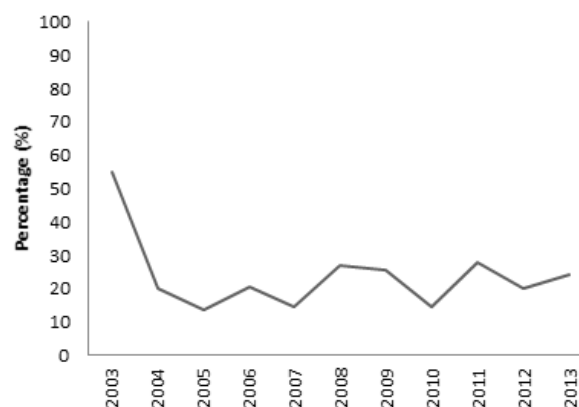
	2003	2004	2005	2006	2007	TOTAL	%
None	12	72	34	28	29	175	47.62
H	1	4	8	6	4	23	6.59
R	28	10	0	2	1	41	9.16
E	0	6	1	1	1	9	2.01
S	3	4	1	2	0	10	3.11
Z	0	0	0	0	2	2	2.38
H-R	5	5	3	3	6	22	6.96
H-E	2	2	1	0	0	5	1.1
H-S	1	2	5	1	3	12	3.11
R-E	0	4	6	0	0	10	1.83
R-S	2	3	0	1	0	6	1.1
E-S	2	0	0	0	0	2	0.37
H-Z	0	0	0	0	0	0	0.92
R-Z	0	0	0	0	0	0	0
E-Z	0	0	0	0	0	0	0
S-Z	0	0	0	0	0	0	0
H-R-E	1	0	1	6	3	11	2.56
H-R-S	0	3	2	1	3	9	2.38
R-E-S	0	0	0	0	0	0	0
H-E-S	1	2	6	0	0	9	1.83
H-R-Z	0	0	0	0	0	0	0.92
H-E-Z	0	0	0	0	0	0	0.18
H-S-Z	0	0	0	0	1	1	0.73
R-E-Z	0	0	0	0	0	0	0
R-S-Z	0	0	0	0	0	0	0
E-S-Z	0	0	0	0	0	0	0
H-R-E-S	0	2	6	2	1	11	2.75
H-R-E-Z	0	0	0	0	0	0	0.37
H-R-S-Z	0	0	0	0	0	0	1.47
H-E-S-Z	0	0	0	0	0	0	0
R-E-S-Z	0	0	0	0	0	0	0
H-R-E-S-Z	0	0	0	0	1	1	0.55

H=Isoniazid R=Rifampicin E=Ethambutol S=Streptomycin Z=Pyrazinamide

However, it started to decline again in 2008 at 26.92%. And despite the initial increase in 2009-2010, it eventually reached only 37.93% in 2013. Overall, there was a decrease in pansusceptibility from 48.75% in 2003-2007 to only 46.62% from 2008-2013. This was due to the increasing trend of both single and multi-drug resistance, particularly from 2009 to 2013. (Figure 1 and Table VII)

Trends of Single-Drug Resistance Rates in 2003-2007^a, 2008-2013

The trends of single-drug resistance from 2003-2013 shows an initially high percentage of resistance in a

**Figure 1: Overall trends of susceptibility, single-drug resistance and multiple-drug resistance from 2003-2013****Figure 2: Trends of single drug susceptibility and resistance pattern from 2003-2013**

single-drug, which dropped from 55% to 14.55% in 2007. This slightly increased to 26.92% in 2008 but remained stable until 2013 at 24.14%. (Figure 2) Individually, the highest proportion of resistance in 2003-2007 came from rifampicin, but shifted to isoniazid in 2008-2013, as previously seen in Table V.

Trends of Two-Drug Resistance Rates in 2003-2007^a, 2008-2013

There was a stable two-drug resistance pattern in 2003 to 2007 (9.43% to 20.68%), highest with H-R combination (6.96%). However, it began to increase in 2008 (30.77%), then slowly declined until there was no two-drug resistance noted in 2013. (Figure 3)

Trends of Three-Drug Resistance Rates and Four-Drug Resistance Rates from 2003-2007^a, 2008-2013

There was an initial increase in three-drug resistance rate in 2003-2007, highest in 2006 at 13.21%. However, the trend began to decline in 2008-2012, with no noted drug resistance in 2011, but again showed an increasing rate in 2013 at 24.14%. H-R-E resistance (2.56%) was highest in 2003-2007 compared to H-R-Z (2.57%) in 2008-2013. (Figure 4)

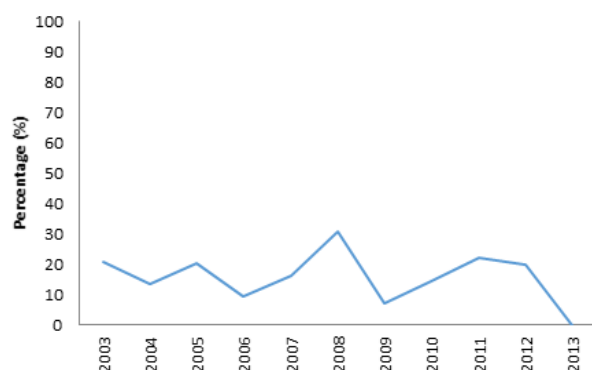


Figure 3: Trends of two drug susceptibility and resistance pattern from 2003-2013

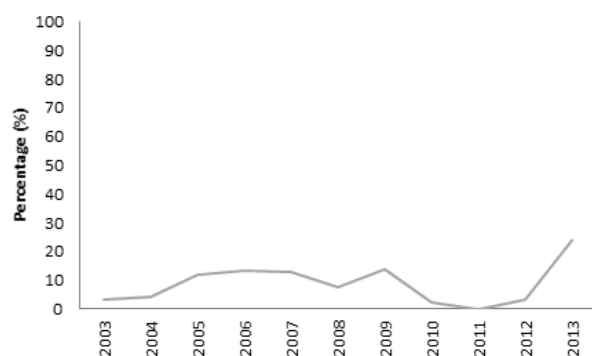


Figure 4: Trends of three drug susceptibility and resistance pattern from 2003-2013

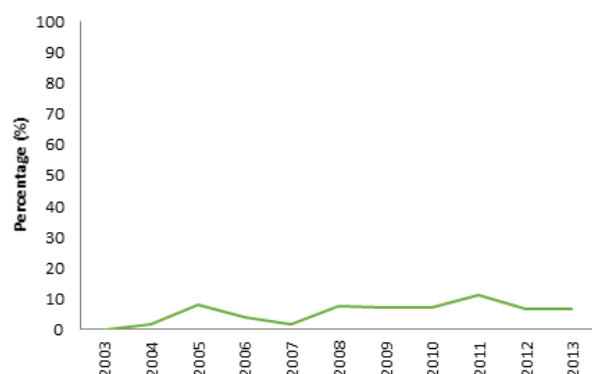


Figure 5: Trends of four drug susceptibility and resistance pattern from 2003-2013

Finally, there is an increasing trend of resistance to four-drug combination from 2003 to 2007, which continued to rise from 2008 to 2013. The combination with the highest resistance was H-R-E-S in 2003-2007, compared with H-R-Z-S in 2008-2013. (Figure 5)

There was almost no panresistance noted from 2003 to 2013, with few isolated cases in 2007 (n=1) and 2013 (n=2).

Discussion

Inhalation of the infectious MTB droplet nuclei leads to one of four possible outcomes, namely: immediate clearance of the organism, latent infection, onset of active disease (primary disease), or active disease after several years (reactivation disease).⁸

The long latent period makes it difficult for the different strains of the organism to interact and exchange genetic information. Unlike other microorganisms which can transmit antibiotic resistance through genetic transfer, in MTB, most resistance are acquired through chromosomal mutations of drug target genes.⁹ The probability of a mutation is also proportional to the bacillary load. Likewise, there is a high likelihood that a mutant microorganism is simultaneously resistant to two or more drugs.¹⁰

Resistances to the different first line anti-tuberculosis drugs have different molecular mechanisms. Isoniazid, which is a pro-drug, has to be acted upon by a catalase peroxidase encoded by *katG* gene.¹¹ The activated drug then disrupts mycolic acid biosynthesis and this eventually results in loss of cellular integrity, causing bacterial death.¹² Thus, the decrease in or loss of catalase activity results in isoniazid resistance. MTB, is able to adapt to the loss of *katG* function by overexpressing a protein, *ahpC*, which can detoxify organic peroxides.¹³ In studies made to determine the causes of isoniazid resistance, the *inhA* locus and *KasA* were discovered. These proteins are involved in the synthesis of mycolic acids and the mutations were found in the promoter regions, or less commonly in the genes that encode these proteins.¹⁴ Mutations in *inhA* also cause resistance to Ethionamide which is a structurally related second-line drug.¹⁵ Mutations in *ndh*, which encodes NADH dehydrogenase, also causes isoniazid resistance through decrease in activity of *ndh* which causes an increase in NADH/NAD ratio which compete with the binding of the activated isoniazid to the target *inhA* or promote its displacement from *inhA*.¹⁶

Resistance to rifampicin arises due to mutations in the beta subunit of RNA polymerase encoded by the gene *rpoB*.⁹ This mutation results in the inhibition of elongation of messenger RNA. Rifampicin has early bactericidal effect on metabolically active MTB and shows excellent late sterilizing action on semidormant organisms with slow metabolic activity. Resistance to rifampicin alone is rare compared to monoresistance to isoniazid. But rifampicin resistance can be detected in isoniazid resistant strains and thus, rifampicin resistance detection can be used as an alternate marker for multi-drug resistance.¹⁷

Table V: MTB positive cultures susceptibility and resistance rates from 2008-2013

	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	TOTAL	%
None	12	72	34	28	29	7	20	25	7	15	11	260	47.62
H	1	4	8	6	4	1	6	2	1	0	3	36	6.59
R	28	10	0	2	1	4	0	2	1	2	0	50	9.16
E	0	6	1	1	1	0	1	1	0	0	0	11	2.01
S	3	4	1	2	0	2	2	1	1	1	0	17	3.11
Z	0	0	0	0	2	0	2	0	2	3	4	13	2.38
H-R	5	5	3	3	6	4	3	4	4	1	0	38	6.96
H-E	2	2	1	0	0	1	0	0	0	0	0	6	1.1
H-S	1	2	5	1	3	3	0	2	0	0	0	17	3.11
R-E	0	4	6	0	0	0	0	0	0	0	0	10	1.83
R-S	2	3	0	1	0	0	0	0	0	0	0	6	1.1
E-S	2	0	0	0	0	0	0	0	0	0	0	2	0.37
H-Z	0	0	0	0	0	0	0	0	0	5	0	5	0.92
R-Z	0	0	0	0	0	0	0	0	0	0	0	0	0
E-Z	0	0	0	0	0	0	0	0	0	0	0	0	0
S-Z	0	0	0	0	0	0	0	0	0	0	0	0	0
H-R-E	1	0	1	6	3	0	1	0	0	0	2	14	2.56
H-R-S	0	3	2	1	3	2	1	0	0	0	1	13	2.38
R-E-S	0	0	0	0	0	0	0	0	0	0	0	0	0
H-E-S	1	2	6	0	0	0	0	0	0	0	1	10	1.83
H-R-Z	0	0	0	0	0	0	2	0	0	1	2	5	0.92
H-E-Z	0	0	0	0	0	0	1	0	0	0	0	1	0.18
H-S-Z	0	0	0	0	1	0	1	1	0	0	1	4	0.73
R-E-Z	0	0	0	0	0	0	0	0	0	0	0	0	0
R-S-Z	0	0	0	0	0	0	0	0	0	0	0	0	0
E-S-Z	0	0	0	0	0	0	0	0	0	0	0	0	0
H-R-E-S	0	2	6	2	1	2	1	0	1	0	0	15	2.75
H-R-E-Z	0	0	0	0	0	0	0	0	0	1	1	2	0.37
H-R-S-Z	0	0	0	0	0	0	2	3	1	1	1	8	1.47
H-E-S-Z	0	0	0	0	0	0	0	0	0	0	0	0	0
R-E-S-Z	0	0	0	0	0	0	0	0	0	0	0	0	0
H-R-E-S-Z	0	0	0	0	1	0	0	0	0	0	2	3	0.55
	58	119	74	53	55	26	43	41	18	30	29	546	100

Table VI: Prevalence of Drug Resistance of the First-Line Drugs (Either alone or in combination) from 2003-2007⁶ and 2008-2013

	2003-2007 ⁶	2008-2013	2003-2013
Isoniazid	104 (28.97%)	72 (38.50%)	176 (32.23%)
Rifampicin	107 (30.92%)	53 (28.34%)	160 (29.30%)
Ethambutol	58 (16.60%)	16 (8.56%)	74 (13.55%)
Streptomycin	61 (16.11%)	34 (18.19%)	95 (17.40%)
Pyrazinamide	4 (1.11%)	37 (19.79%)	41 (7.51%)

Pyrazinamide is an analog of nicotinamide which is only active in acidic pH.¹⁸ This became an important component of short-course therapy because of its activity against semidormant bacilli and has strong synergy with isoniazid and rifampicin.¹⁹ Pyrazinamide

is a pro-drug that is activated by mycobacterial enzyme pyrazinamidase into its active form pyrazinoic acid. Thus, a mutation in the *pncA* gene or in the promoter region of the gene which encodes for pyrazinamidase causes resistance.²⁰

Ethambutol interferes with the biosynthesis of the cell wall arabinogalactan and is active against multiplying bacilli.¹⁷ Resistance to ethambutol occurs due to point mutations in the *embCAB* operon which encodes homologous arabinosyl transferase enzymes.²¹

Streptomycin is an aminocyclitol glycoside antibiotic and resistance to this drug is mostly due to mutations in *rrs* and *rpsL*, which produce alterations in the streptomycin binding site.¹⁷ Low level resistance to streptomycin has been found in mutations in *gidB*

Table VII: Susceptibility and drug resistance pattern from 2003-2007⁶, 2008-2013

Percentage (%)	2003	2004	2005	2006	2007	2003-2007	2008	2009	2010	2011	2012	2013	2008-2013
Single Drug Resistance	55.17	20.17	13.51	20.75	14.55	23.68	26.92	25.58	14.63	27.78	20	24.14	22.46
Two-Drug Resistance	20.68	13.45	20.27	9.434	16.36	15.88	30.77	6.98	14.63	22.22	20	0	14.44
Three-Drug Resistance	3.45	4.20	12.16	13.21	12.73	8.36	7.69	13.95	2.44	0	3.33	24.14	9.09
Four- Drug Resistance	0	1.68	8.11	3.77	1.82	3.06	7.69	6.98	7.32	11.11	6.67	6.90	7.49
Five-Drug Resistance	0	0	0	0	1.82	0.28	0	0	0	0	0	6.90	1.07
Fully Susceptible	20.69	60.50	45.95	52.83	52.73	48.75	26.92	46.51	60.98	38.89	50	37.93	47.62

which encodes for a conserved 7-methylguanosine methyltransferase specific for the 16S rRNA.²²

Ahmad, et al.²³ reported the relative frequency of some of the previously mentioned gene mutations in resistant strains of MTB and these are: 50-95% for *katG*, 5-43% for *InhA*, 90-95% for 81-bp rifampicin resistance determining region (RDDR) in the *rpoB*, 2-5% for the N-terminal region of the *rpoB*, 68-95% for *pncA*, 35-89% for *embB*, 20-81% mutation for *rpsL*, and 10-30% for *rrs*. The molecular detection of rifampicin resistance is most helpful because it has the highest frequency of mutation and 80-95% of rifampicin resistant organisms are also resistant to isoniazid and thus, MDR-TB strains can be easily identified.¹⁷

Prompt detection of drug resistance to MTB is essential for effective control of tuberculosis. Compared to the study done six years ago⁶, wherein 359 out of 1,400 specimens were positive for culture and DST, there is a decline in the total number of specimens submitted for DST from 2008 to 2013. In the current study, out of the 4,881 submitted specimens, only 187 specimens were sent for sensitivity testing. This may be due to the increasing cost of laboratory services as well as the inability of thorough follow-up of the results to be able to have the specimen be tested for sensitivity studies because of the long waiting period for MTB to grow.

The susceptibility rate showed a decreasing trend at 48.75% in 2003-2007⁶ to only 46.62% in 2008-2013. This was reflected in the increase in both single and multi-drug resistance rates. The multi-drug resistance rate of 29.12% in our institution is significantly higher than the results of the WHO¹ and Philippine Drug Resistance Surveillance². The resistance rate to any drug of 52.38% is also higher than the 19.33% combined resistance rate to any drug among new and retreatment cases in the cited national survey. These discrepancies may primarily be attributable to a selection bias of requesting DST, mostly among presumptive drug-resistant TB cases only, in actual clinical practice; and with USTH being known, especially among its medical school graduates and former trainees, as a referral facility for difficult-to-manage cases. In addition, the predominantly rural (68.4%) study population and the 757 missing DST data for rifampicin and isoniazid in the national drug

resistance surveillance may also contribute to the variance. But just the same, the significant resistance rates are definitely a cause of concern for all TB health service providers.

With isoniazid and rifampicin as primary essential anti-TB medications used in both intensive and continuation phases, it is unfortunate that resistance rates for these vital drugs are the most frequently documented — whether single or combined, and in combination with ethambutol and/or streptomycin. This forebodes challenging times in TB control with the increased need for second-line drugs - which are more toxic, more expensive and, consequently, more difficult to use without proper training.

Considering the release of several editions of local and international guidelines, the introduction of fixed dose combination (FDC) drugs, the promotion of Directly Observed Treatment — Short Course (DOTS) strategy, even among the private sector, and the strengthening of the National TB Control Program during the last two decades, drug-resistant TB remains to be a significant problem for the country. The introduction and expanding access to rapid diagnostic tests for detecting drug-resistant strains of TB can hopefully facilitate its early recognition and proper management.

Conclusion

Despite the variety of measures and programs being implemented for the detection and treatment of MTB, as well as the availability of FDCs, the overall MTB resistance rate from 2003 to 2013 at the USTH, as a referral facility, is still increasing. Resistance to the first line drugs used for both initiation and maintenance phases of treatment (H and R) as well as the two-drug combinations (H-R), three-drug combinations (H-R-E, H-R-S) and four-drug combinations (H-R-E-S) are still high. Failure to recognize these existing drug resistance patterns can further propagate MDR-TB strains, which are more difficult to control and treat, and requires the more expensive and more toxic second-line drugs. Likewise, the importance of microbiologic identification and drug sensitivity testing, using diagnostic modalities that can detect both MTB complex and MDR-TB strains in the least amount of time, at the start of therapy, should be emphasized

especially in patients at risk for drug resistance, since it is a useful guide in individual patient management.

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