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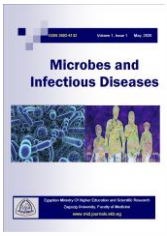

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Original article

Drug resistance trends of *Mycobacterium tuberculosis* before and after the COVID-19 pandemic in an Egyptian Cairo University tertiary-care hospital

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ABSTRACT

Background: Tuberculosis (TB) is a global public health concern, associated with high mortality worldwide. The COVID-19 pandemic interrupted diagnostic and therapeutic services. These factors could have an effect on the rates of drug resistance of *Mycobacterium tuberculosis* (MTB). We aimed to compare the rates of drug resistance of MTB to streptomycin, isoniazid, rifampin, and ethambutol (SIRE) drugs before and after the pandemic. **Methods:** This cross-sectional study was conducted with a total of 100 MTB isolates, equally divided into 2 groups: Group (A) included isolates from samples collected in 2019 (before COVID-19) and Group (B) included isolates for samples collected in 2020-2021 (after COVID-19 pandemic). We tested the drug susceptibility of all MTB isolates by the automated proportion method using the BD BACTEC MGIT 960 SIRE Kit, operated on the MGIT 960 instrument system. **Results:** The overall resistance of MTB isolates to the SIRE drugs was recorded at rates of 21%, 15%, 18% and 11%, respectively. There was an observed decrease in the susceptibility to SIRE anti-TB drugs from rates of 90%, 88%, 84%, 100% to 68%, 82%, 80%, 78%, respectively, with significant increase in total multidrug-resistance (MDR) rates in Group (B) compared to Group (A) MTB isolates (P< 0.0001). **Conclusion:** Our study revealed higher rates of multidrug-resistance in Group B (after the COVID-19 pandemic) compared to Group A (before pandemic) MTB isolates. Control measures are urgently required with raising awareness of physicians and building laboratory capacity to detect MDR- MTB.

Introduction

Infection with *Mycobacterium tuberculosis* (MTB) is a high global public health threat as the microorganism infects one-third of the world population, and is among the top 10 leading causes of death all over the world. According to the WHO report (2023) globally, tuberculosis (TB)- infected patients were estimated at 10.6 million in 2022, and death from TB occurred in 1.30 million [1]. TB is more prevalent in low and middle-income countries because it is linked to poverty, inadequate

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sanitation, and is easily transmitted from person to person [2]. Moreover, TB diagnosis and treatment constitute a significant burden on the healthcare system and catastrophic costs among the TB households [3].

Globally, the incidence of TB reduced steeply from $184/10^5$ in 2000 to $129/10^5$ in 2020, and then it showed a slight increase to reach $134/10^5$ in 2021. Similarly, the incidence of TB showed a remarkable reduction in Egypt; from 26 cases per 10^5 in 2000 to 10 per 10^5 in 2021. This may be due to improvement in treatment success rates from 69 to 86% from 2000 to 2020 [2].

In spite of the global control measures that decreased the incidence of TB in many countries, resistance of MTB to anti-TB drugs emerged leading to drug resistant (DR) and multi-drug resistant (MDR) isolates of MTB, which contributed to therapeutic failure [1,2].

Among the significant risk factors for treatment failure and the subsequent emerging drug resistance were unavailable diagnostics, decreased access to appropriate anti-TB drugs, interrupted therapy [4].

Treatment for people diagnosed with rifampicin-resistant TB (RR-TB), isoniazid-resistant TB and MDR-TB requires regimens that include second-line drugs, such as bedaquiline and fluoroquinolones; these regimens are more expensive and cause more side-effects than first-line treatments for drug-susceptible TB, and less successful in treatment [5].

In Egypt, TB is still considered a significant national health problem [4], with variable drug resistance rates as reported in previous studies [6-9]. Globally, the COVID-19 pandemic caused disruptions to the provision of and access to TB diagnostic and treatment services, which are among the significant risk factors for treatment failure and the subsequent emerging drug resistance [4].

The pandemic was estimated to have caused an increase of about 100 000 in the global number of TB deaths between 2019 and 2020 [10]. Moreover, COVID-19 infection among TB patients was associated with unfavorable effects due to the interaction of the two infections, and the decreased access to anti-tuberculosis treatment [11,12]. The WHO estimated an increase of 4.5% in the incidence of TB from 2020, and drug-resistant TB also increased by 3% between 2020 and 2021 [13].

In this study, we aimed to assess the phenotypic susceptibility and the different multidrug-resistance phenotypes of the MTB isolates to first-line anti-TB drugs before and after the COVID-19 pandemic.

Material and Methods

Study design

The present study is a cross-sectional study, conducted with a total number of 100 MTB isolates from positive culture sputum samples for patients clinically suggested of pulmonary tuberculosis addressing Kasr- AlAiny Cairo University Hospital. The tested MTB isolates were assigned chronologically to two groups of samples based on the period of collection. Group (A) included MTB isolates (n=50) for samples that were collected in the period (T1) from January to December 2019; before COVID-19 pandemic, and Group (B) enrolled MTB isolates (n=50) from samples that were collected from April 2020 to December 2021 (T2) (after the pandemic). All samples for the MTB isolates were already subjected to full mycobacteriology work up, as summarized in **Figure 1**, including smear microscopic examination, culture on Löwenstein–Jensen (LJ) slants and GeneXpert assay, as detailed below [14 -16]. All MTB isolates were sub- cultured to perform phenotypic drug susceptibility to the first line anti-TB drugs (streptomycin, isoniazid, rifampin and ethambutol), in order to compare the MTB drug resistance profile in both study periods (before and after COVID-19 pandemic). All laboratory procedures were carried out under strict biosafety measures in biosafety level (BSL3) Mycobacteriology Laboratory of Cairo University Hospital, Egypt.

Specimen Preparation

Non-sterile specimens were decontaminated with NALC/ NaOH solution composed of 1% N-acetyl-L-cysteine (NALC), 4% sodium hydroxide (NaOH), and 2.9% sodium citrate. The NALC/ NaOH solution was shaken with the specimen in screw capped centrifuge tubes, then centrifuged at 15000xg (13000 rpm) for 5 minutes for concentration. After aseptically decanting the supernatant, sterile phosphate buffer (1.5 ml) was added to resuspend the sediment. The sediment was used for microscopic examination of direct smears, culture on Lowenstein-Jensen (LJ) medium slants and for Xpert assay [14].

Smear microscopy and GeneXpert assay

All sputum samples were subjected to direct smear microscopy and GeneXpert assay for molecular MTB detection. All samples were prepared from all clinical specimens and stained with Ziehl-Neelsen (ZN) stain and examined microscopically for acid-fast bacilli [15]. The specimens were directly tested using GeneXpert® MTB/RIF assay (Cepheid, Sunnyvale, USA) for automated simultaneous fast detection of MTB and Rifampicin (R) resistance. The assay is a real-time semi-nested PCR system. The assay is a WHO-recommended rapid diagnostic test to achieve early and accurate diagnosis [3]. The test procedure was carried out following the manufacturer's instructions.

Isolation of MTB and phenotypic Drug susceptibility testing (DST)

All sputum specimens were cultured on LJ slants that were manually prepared and quality checked for sterility and performance. LJ slants were inoculated with a volume of 0.2 ml of the prepared specimens, incubated at 37ºC and were inspected weekly over a period of 8 weeks for mycobacterial growth [16]. MTB isolates were identified based on the rate of growth, colony morphology and ZN stain microscopy. All MTB isolates were stored at -80 ºC and sub-cultured on LJ medium slants to obtain fresh colonies for phenotypic drug susceptibility testing. All Group (A) and Group (B) MTB isolates were tested for their phenotypic antimicrobial susceptibility to streptomycin, isoniazid, rifampin and ethambutol (SIRE) first- line anti-TB drugs using the BD BACTEC MGIT 960 SIRE Kit operated on the MGIT 960 instrument system (Becton Dickinson, Sparks, Md., USA) following the manufacturer's instructions [17]. MDR-TB is defined as resistance to isoniazid and rifampicin, with or without resistance to one or more of the first line anti-TB drugs [6, 18].

Ethical approval

The study obtained an ethical approval from the Research Ethics Committee of the Faculty of Medicine, Cairo University (approval number: N-53-2019), and was conducted according to Helsinki Declaration principles. All isolates were de-identified to maintain patient privacy.

Statistical analysis

Data were presented in the form of numbers (N) and percentages (%). Significant difference between compared study groups was assessed using Chi-square (X^2) test, where P values less than 0.05 were considered significant. Statistical calculations were done using the SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) V.15.

Results

In the present study, total 100 MTB isolates sorted into Group (A) (n=50) and Group (B) (n=50), were tested for their phenotypic susceptibility to SIRE anti-TB drugs, to compare MTB drug resistance profiles between 2 time periods (T1: year 2019, and T2: year 2020-2021). Among total MTB-positive culture sputum samples, 67 were smear-positive and 98 were GeneXpert-positive.

Table 1 illustrates the comparative phenotypic susceptibility results of Group A and Group B MTB isolates to the SIRE anti-TB drugs. The overall resistance rates of total MTB isolates of both groups to S (streptomycin), INH (isoniazid), RF (rifampin) and E (ethambutol) SIRE anti-TB drugs were 21%, 15%, 18% and 11%, respectively. Pan-susceptibility to all SIRE drugs was demonstrated in 71% of total isolates, with the proportions of 40/50 and 31/50 among Group (A) and Group (B) isolates, respectively. Among Group(A) isolates, the least susceptibility rates were recorded for RF (84%), followed by isoniazid (88%), with corresponding resistance rates of 16% and 12%, respectively. Group (B) isolates recorded the least susceptibility rates to S (68%) and RF (80%), with corresponding resistance rates of 32% and 20%, respectively. Ethambutol showed no resistance in Group (A) isolates, however recorded resistance rate of 22% in Group (B) isolates.

By comparing the Group (A) and Group (B) isolates along the 2 periods of the study, it was noted that the susceptibility rates to all tested anti-TB drugs were reduced with concomitant increase in the rates of resistance in Group B isolates (2020-2021) compared to Group A (2019), as revealed in **Table 1**. Different patterns of multidrug resistance were observed among Group A and B isolates in the form (INH+RF), (INH+RF+E) and (INH+RF+S+E) with rates of 12%, 14% and 14%, respectively among total MTB isolates (n=100), and rates of 30%, 35% and 35% among total MDR isolates (n=40) **(Table 2)**. A trend of significant increase in total multidrug-resistance rates was noted in Group (B) compared to Group (A) isolates ($P< 0.0001$). The resistance phenotypes of (INH+RF), (INH+RF+E) and (INH+RF+S+E) were rising from

6%, 6% and 4% in Group (A) to 18%, 22% and 24% in Group (B) isolates **(Table2 and Figure 2)**. Significant differences were found for increased resistance to Streptomycin, Ethambutol, as well as MDR patterns of (INH+RF+E) and (INH+RF+S+E) between Group (A) and Group (B).

Table 1. Drug susceptibility test results of the 2 groups of MTB isolates.

Group of MTB isolates	**Group A N=50**		**Group B N=50**		**P- value**	**Total isolates N=100**	
Time period (Years of isolation)	**T1 (2019)**		**T2 (2020-2021)**			**T1+T2 (2019-2021)**	
Drugs	**S (%)**	**R (%)**	**S (%)**	**R (%)**		**S**	**R**
Streptomycin (S)	45 (90)	5 (10%)	34 (68)	16 (32%)	0.014*	79	21
Isoniazid (INH)	44 (88)	6 (12%)	41 (82)	9 (18%)	0.57	85	15
Rifampicin (RF)	42 (84)	8 (16%)	40 (80)	10 (20%)	0.79	82	18
Ethambutol (E)	50(100)	0 (0%)	39 (78)	11 (22%)	0.001*	89	11
Pan-susceptible to SIRE	40 (80%)		31 (62%)		-	71	

*Significant P- value is < 0.05

Table 2. Rates of different patterns of multidrug resistance among both MTB isolates groups.

Patterns of MTB drug resistance	**Group A, T1 N =50**	**Group B, T2 N =50**	**P- value**	**Total isolates N=100**
MDR (INH+RF)	3 (6%)	9 (18%)	0.12	12
MDR (INH+RF+E)	3 (6%)	11 (22%)	0.04*	14
MDR (INH+RF+S+E)	2(4%)	12 (24%)	0.02*	14
Total MDR	8 (16%)	32 (64%)	<0.0001*	40

MDR: multidrug-resistant, *Significant P- value is < 0.05

Figure 1. Laboratory workflow for detection of *Mycobacterium tuberculosis*.

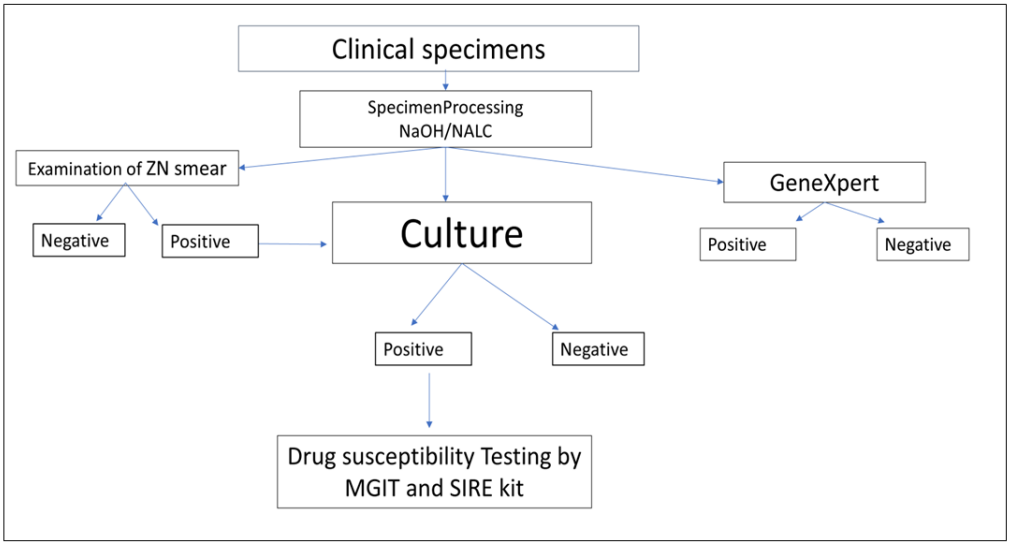
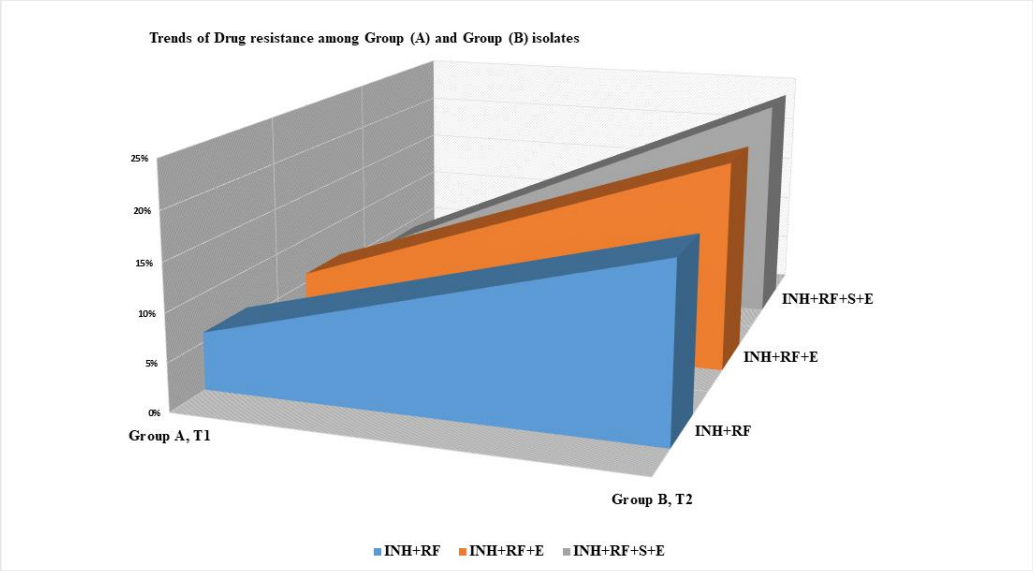


Figure 2. Trends of multidrug resistance among Group (A) and Group (B) MTB isolates before (T1) and after (T2) COVID-19 pandemic.



Discussion

Although the global response to COVID-19 has successfully controlled the spread of the virus, yet it has caused serious disruptions to the control programs for other major diseases. For TB in particular, lockdowns on society severely curtailed diagnosis and case finding [19] and potentially the availability of drugs [20-22]. Accordingly, this raised the assumption of potential consequent increase in TB drug resistance. Hence, we aimed to compare phenotypic first- line drugs susceptibility profiles of 100 MTB isolates over two time periods (before and after COVID-19 pandemic). A modelling analysis commissioned by the STOP TB Partnership, Geneva, Switzerland, indicates that the COVID-19 pandemic has deeply affected TB prevention, case detection and management [21]; factors inductive of resistance emergence, evolution and spread. The MTB resistance to anti-TB drugs has been a major public health obstacle to achieve global tuberculosis control and eradication [21].

The standard method for diagnosing TB is culturing but unfortunately, it is time consuming. Rapid molecular methods are faster diagnostics. The GeneXpert MTB/RIF was endorsed by the WHO; as a rapid and automated molecular system for simultaneous MTB detection. In our study, MTB was detected by, ZN smear, culture and GeneXpert. Unlike the ZN smear, that encountered positivity only in 67% of positive-culture sputum samples, the GeneXpert managed to detect MTB in 98 out of 100 positive culture sputum samples. This aligns with previous reports from Egypt and other countries, which affirm the superiority of GeneXpert assay in MTB detection, with sensitivity ranging from 90% to 100%. [23-27].

Drug-resistant TB (DR-TB) continues to be a public health threat. Resistance to at least rifampicin and isoniazid among first-line anti TB drugs is defined as MDR-TB. MDR-TB urges the use of second-line anti-TB drugs, which recorded low success rates in treatment ranged from 56-69% (average 59%) [5]. In our study, we tested the susceptibility of MTB isolates to streptomycin (S), isoniazid (I), rifampicin (R), and ethambutol (E) drugs with recorded resistant rates of 21%, 15%, 18% and 11%, respectively and total MDR rate of 40%. In the same context, the overall pan-susceptibility to SIRE drugs was detected in only 71% of the isolates. Generally, the low susceptibility rates to anti-TB drugs in clinical practice is much attributed to therapeutic failure and relapses due to interrupted treatment [1,2]. Compared to our study, earlier studies from Egypt reported lower MDR resistance of MTB at rates ranging 5.5%-10.9% [24-26], and likely were previous reports from other countries (5.4%-12%) [28, 29]. In Saudi Arabia, resistant percentages to SIRE were 5%, 1.6%, 0% and 0%, respectively [8]. Lower resistance rates were also reported from Europe, with an average

MDR rate of 3.7% across European countries [30]. The fore-mentioned studies with low resistance rates were conducted before the era of the COVID-19 pandemic, while in our study half of tested MTB isolates were collected during the COVID-19 pandemic, when tuberculosis patients suffered hardships for reaching out proper diagnosis and treatment, due to the measures of lockdown. This may explain the lower MDR rate (16%) in our study, before the pandemic, which is more comparable to prior studies, than after the pandemic (64%). It is worth to note that a possible explanation to the high resistance rates in our study, may be that MTB isolates could be from patients with previous anti-TB drug intake or failed treatment, as reported in previous studies [25, 31, 32]. Unfortunately, in our study, there was no specific data available on the status of previous drug intake for patients who gave sputum samples, however as for a tertiary- care hospital, where our study was conducted, many of the patients receiving care are those who come for follow up or who do not respond to therapy. Geographical variation also plays a considerable role as high TB burden countries are expected to have higher multidrug-resistance rates [31, 32].

In our study, the dominant patterns of resistance among MDR-MTB isolates (35%, 14/40) were exhibited to INH+RF+S+E, as well as to INH+RF+E. This agreed well with another study from Egypt that reported the highest pattern of MDR resistance for INH+RF+S+E (46.5%) [25]. This is in line with other several reports of conferred resistance to 3-4 anti-TB drugs among MDR isolates [31, 33], supporting the recommendation of starting empirical use of the 4 anti-TB drugs instead of depending on 1 or 2 drugs, until full susceptibility result is released [33].

By comparing the two studied groups, there was an observed decrease in the susceptibility to all SIRE anti-TB drugs from rates of 90%, 88%, 84% and100% to rates of 68%, 82%, 80% and 78%, respectively. Correspondently, there was a significant increase in the overall percentage of MDR in Group (B) isolates (2020-2021) after the COVID-19 pandemic compared to Group (A) (2019) before the pandemic, with significant difference for the phenotypes of INH+RF+E and INH+RF+S+E. This shows the temporal trends towards increased resistance rates, and confirms previous reports about the deleterious effect of the COVID-19 pandemic on the TB control programs. This is in keeping with WHO estimated increase of 4.5% in the incidence of TB from 2020, with increase in drug-resistant TB by 3% between 2020 and 2021 [5], and the global increase of TB incidence to $134/10^5$ in 2021 from $129/10^5$ in 2020 [2]. The findings of our study could reflect the adverse consequences of the COVID-19 on the magnitude of the multidrug- resistance problem of TB, which might be due to the lockdown measures that caused interrupted services of TB diagnosis and treatment. We recommend leveraging the capacity of clinical laboratories for improving MTB detection and drug susceptibility testing, in order to prescribe targeted effective drugs and achieve better control of TB disease. Furthermore, larger scale national multi-center researches covering the entire panel of anti-TB drug susceptibility are encouraged, to address the problem of drug resistant TB more comprehensively in our country.

Conclusion

We report high overall rates of resistance among the MTB isolates, and higher rates of resistance in the isolates collected during and after the COVID-19 pandemic, compared to isolates collected before the pandemic. The COVID-19 pandemic could have contributed to the increased rates of drug resistance. However, it is worth to note, that the small number of isolates and being from only one tertiary center limits our study. In this view, we recommend building the capacity of clinical laboratories for adopting MTB drug susceptibility testing by automated equipment and experienced laboratory staff. We also recommend awareness campaigns on DR in MTB, and advice drug susceptibility testing to prescribe targeted effective drugs that will optimize therapy, and control TB dissemination.

Limitations

Our study was limited by deficient demographic and clinical data of the patients, and lack of detailed information on patient treatment. Furthermore, we performed MTB drug susceptibility testing to isolates from single tertiary-care hospital, and only for first-line anti- TB drugs, without involvement of the second-line, or drugs recently introduced in treatment.

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None

Conflicts of interest

The authors declare that they do not have any conflict of interest.

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