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The Patterns of Conversion to Anti-Tuberculosis Drug Resistance in *Mycobacterium tuberculosis*

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Abstract

The prevalence of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) is increasing. We analyzed the patterns of drug resistance and the days of tracking period of acquiring anti-mycobacterial resistance. From January 2010 to December 2019, drug susceptibility tests (DST) were performed by the absolute concentration method using the Löwenstein-Jensen solid medium and pyrazinamidase activity test (to assess pyrazinamide resistance) in samples from patients who were referred to the Green Cross Laboratories in Yongin. Among the cases that showed resistance to one or more anti-tuberculosis drugs, 55 patients (33.1%) were resistant to isoniazid (INH) at the time of initial referral, and the rates for the development of resistant anti-tuberculosis drugs were ethambutol (EMB) (26.6%), rifampicin (RFP) (21.9%), quinolones (QUI) (21.9%) and pyrazinamide (PZA) (10.9%), in that order. In the cases sensitive to all 10 anti-tuberculosis drugs initially, the development of resistance to INH was the most frequent, seen in 43 patients (7.2%). The average follow-up period was 435.6 days, and the resistance development was observed in the order of INH (7.2%), RFP (3.9%), SM (1.9%), QUI (0.7%), amikacin (AMK) (0.5%), and EMB (0.5%). The conversion of susceptible strains to resistant strains is an important warning sign for the patient, especially in cases of conversion to MDR or XDR. This information would help improve patient care during TB treatment.

Keywords

Anti-tuberculosis drug
Conversion to resistant
Drug susceptibility
Mycobacterium tuberculosis
Tuberculosis

1. Introduction

The World Health Organization (WHO) classifies tuberculosis (TB) as one of the top 10 causes of death worldwide and the deadliest among infectious diseases [1]. In South Korea, the total number of TB patients in 2020 was 25,350 (49.4 per 100,000 population), which was

approximately 83.7% lower compared to the previous year when there were 30,304 patients (59.0 per 100,000 population). However, South Korea still has the highest incidence rate of TB among the member countries of the Organization for Economic Cooperation and Development (OECD) [2].

TB bacteria grow very slowly, and therefore, the treatment of TB typically involves the simultaneous use of 3–4 different anti-TB drugs to prevent the development of drug resistance when using only 1–2 drugs [3]. Currently, there are challenges in TB treatment, as multidrug-resistant tuberculosis (MDR-TB) has emerged, which is resistant to drugs such as rifampin and isoniazid [4], and extensively drug-resistant tuberculosis (XDR-TB), which is resistant to one or more fluoroquinolone drugs in addition to isoniazid and rifampicin [5]. There have also been reports of totally drug-resistant tuberculosis (TDR-TB), which shows resistance to all first-line and second-line anti-TB drugs [6]. These drug-resistant forms of TB present significant challenges in tuberculosis treatment [7].

MDR-TB can result from acquired resistance due to inappropriate drug use and prescription, or primary resistance caused by initial infection with MDR-TB strains. Therefore, to reduce the prevalence of MDR-TB, successful initial treatment without acquiring resistance is crucial. Patients diagnosed with bacteriologically or clinically confirmed TB who have not previously received TB treatment or have received treatment for less than one month are classified as new patients. However, there is a lack of research on the pattern of acquired drug resistance by phenotypic drug susceptibility testing (DST) in the same patients after diagnosis. Moreover, TB treatment involves the simultaneous administration of various anti-TB drugs for an extended period of 6 months or more, which can lead to side effects, making it challenging to identify the specific drug responsible for the side effects.

This study aims to analyze the transition from drug susceptibility to drug resistance in patients who initially showed resistance to one or more anti-TB drugs and those who were susceptible to all anti-TB drugs, in the same individuals. The goal is to prevent the development of drug resistance and promptly diagnose drug resistance, ensuring that patients receive appropriate treatment from a public health perspective and minimize its impact on society.

2. Materials and methods

2.1. Subjects

This study included patients who had undergone anti-TB drug susceptibility testing at the Green Cross Medical Foundation over a period of 10 years, from 2010 to 2019. Patients who had tested at least twice with an interval of 90 days or more between the first and subsequent tests were recruited. The drug susceptibility testing included primary anti-TB drugs used in initial tuberculosis treated due to their high effectiveness and minimal adverse effects, such as isoniazid (INH), rifampin (RFP), ethambutol (EMB), pyrazinamide (PZA), rifabutin (RBT), streptomycin (SM), amikacin (AMK), kanamycin (KM), capreomycin (CPM), ofloxacin (OFX), levofloxacin (LEV), moxifloxacin (MXF), prothionamide (PTH), cycloserine (CS), para-aminosalicylic acid (PAS), totaling 15 different anti-TB drugs. A total of 10 anti-TB drugs were investigated, including RFP, RBT, AMK, KM, CPM, OFX, LEV, MXF, and quinolone (QUI), since they belong to the same anti-TB drug class, particularly RFP, AMK and QUI as the resistance patterns of these drugs are similar. If patients had undergone drug susceptibility testing multiple times and showed a transition from susceptibility to resistance for one or more anti-TB drugs in the first test, they are included in the study. However, results showing resistance changes after the second test were excluded from the study.

2.2. Methods

The drug susceptibility testing was conducted using the

absolute concentration method recommended by the World Health Organization (WHO) on the Löwenstein-Jensen solid medium. Bacterial isolates cultured on solid or liquid medium were diluted with sterilized phosphate buffer saline (PBS) to achieve a bacterial turbidity of McFarland No. 1.0. These diluted bacterial suspensions were inoculated at a 1:10 ratio onto media without drugs (control media) and media with each specific drug (drug media). After incubation at 35–37°C for four weeks, resistance was determined if there were more than 10 colonies on drug media as compared to control media. The drug types and decision criteria for the 15 anti-TB drugs were as follows: RFP 40 μg/mL, INH 0.2 μg/mL, EMB 2.0 μg/mL, RBT 20 μg/mL, SM 10 μg/mL, AMK 30 μg/mL, KM 30 μg/mL, CPM 40 μg/mL, OFX 4.0 μg/mL, LEV 2.0 μg/mL, MXF 1.0 μg/mL, PTH 40 μg/mL, CS 30 μg/mL, and PAS 1.0 μg/mL. Pyrazinamide (PZA) susceptibility was determined using the pyrazinamidase method [8,9].

3. Result

3.1. Distribution of anti-TB drug resistance during initial treatment

Among 760 sputum specimens requested from 118 medical institutions nationwide for initial anti-TB drug susceptibility testing, 166 cases (R group) showed resistance to one or more of the 10 anti-tuberculosis drugs, while 594 cases (S group) were susceptible to all 10 anti-TB drugs. The annual case distribution is shown in **Figure 1**. The mean age of the R group was 52.7 ± 15.8 years, while the mean age of the S group was 55.8 ± 17.6 years, with no significant difference in age between the two groups.

Among the 166 cases in the R group, 62 cases (37.3%) experienced a transition from susceptibility to resistance in one or more anti-TB drugs, with a follow-up period ranging from 95 days to 2,160 days and an average of 375.3 ± 345.2 days. Among the 594 cases

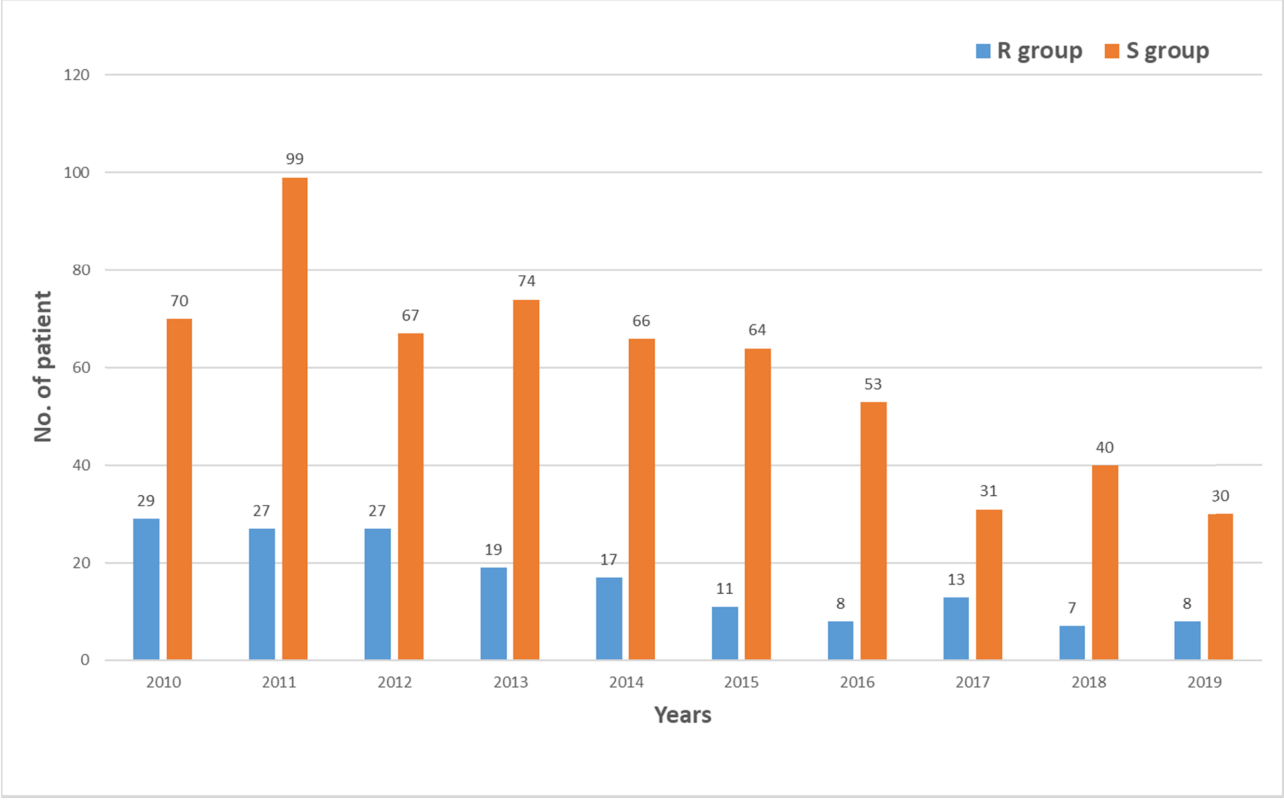

Figure 1. Distribution of anti-TB drug resistance during primary treatment by year. Abbreviation: R group, cases of resistance to one or more anti-TB drugs; S group, cases of susceptibility to all anti-TB drugs.

in the S group, 56 cases (9.4%) exhibited a transition from susceptibility to resistance in one or more anti-TB drugs, with a follow-up period ranging from 97 days to 1,862 days and an average of 453.6 ± 401.4 days.

3.2. Frequency and average follow-up period of drug resistance transition in the R group

Out of the R group, 62 patients (37.3%) exhibited drug resistance to one or more anti-TB drugs, with 48 patients (28.9%) having resistance to a single drug. The results of drug resistance and the average follow-up period for the R group were as follows: AMK 1 case (0.6%), 206.0 days; EMB 9 cases (5.4%), 344.2 days; INH 4 cases (2.4%), 222.2 days; PAS 1 case (0.6%), 202.0 days; PZA 10 cases (6.2%), 249.8 days; QUI 9 cases (5.4%), 420.4 days; RFP 8 cases (4.8%), 351.9 days; SM 2 cases (1.2%), 246.0 days; and PTH 4 cases (2.4%), 355.5 days, showing resistance transition. Among the R group patients, 10 patients (6.0%) exhibited resistance transition to two drugs, with the combinations of EMB and PZE in 1 case (0.6%), 482.0 days; EMB and QUI in 1 case (0.6%), 316.0 days; EMB and PTH in 2 cases (1.2%), 98.0 days; INH and QUI in 1 case (0.6%), 946.0 days; INH and RFP in 1 case (0.6%), 21.0 days; RFP and EMB in 1 case (0.6%), 534. 0 days; RFP and QUI in 2 cases (1.2%), 479.5 days; and SM and PTH in 1 case (0.6%), 355.5 days. There were 3 patients (1.8%) in the R group who exhibited resistance transition to 3 drugs, with one transition to RFP, AMK, and QUI (0.6%) with 236.0 days, one transition to RFP, EMB, and PAS (0.6%) with 2,160.0 days, and one transition to RFP, EMB, and QUI (0.6%) with 251.0 days. Simultaneous resistance transition to 4 anti-TB drugs was observed in 1 case (0.6%) with EMB, AMK, PAS, and PZA, with a follow-up period of 1,115.0 days (**Figure 2**).

Among the R group, INH drug resistance at the time of initial referral had the highest proportion, accounting for 55 patients (33.1%). The drugs with the most extended average follow-up periods for those who experienced resistance transition were EMB (482.7 days), RFP (496.8 days), QUI (396.1 days), and PZA (341.2 days), as shown in **Figure 3**. Furthermore, RFP drug resistance at the time of initial referral was

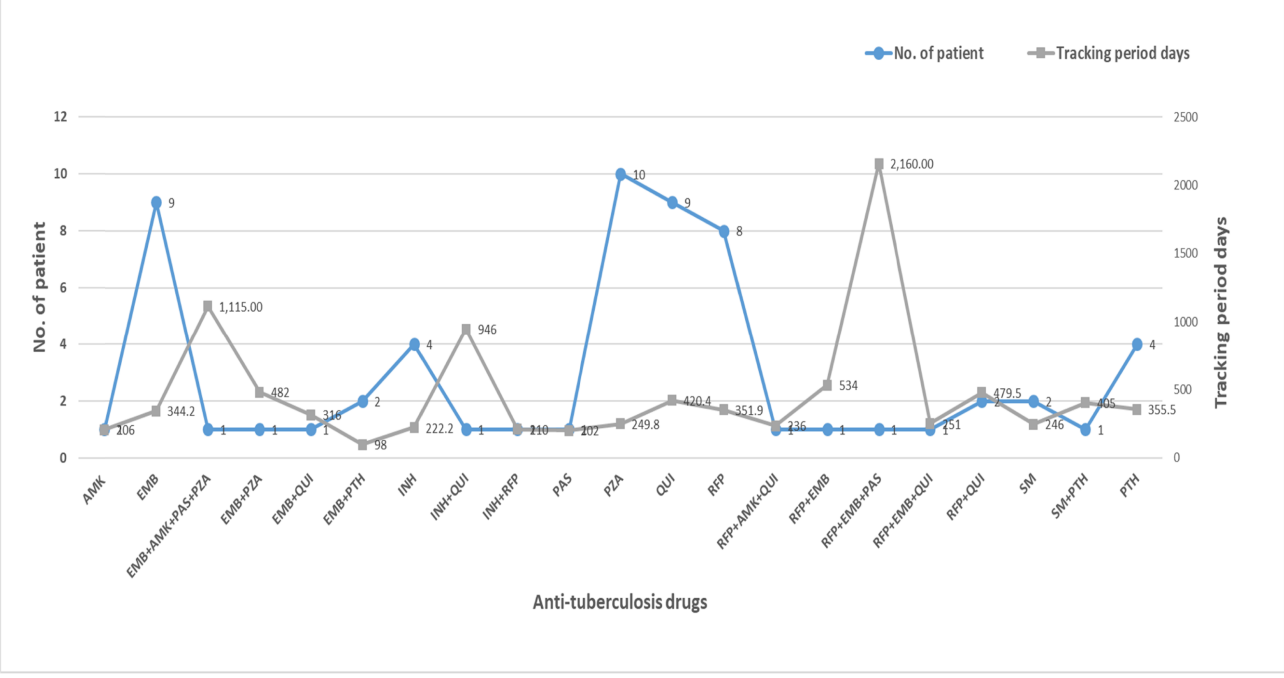

Figure 2. Pattern and average tracking period days of acquiring anti-mycobacterial resistance in R groups (cases of resistance to one or more anti-TB drugs). Abbreviations: INH, isoniazid; RFP, rifampin; SM, streptomycin; QUI, quinolone; AMK, amikacin; EMB, ethambutol; PAS, para-aminosalicylic acid; PZA, pyrazinamide; PTH, prothionamide.

observed in 32 patients (19.3%) with the drugs with the most extended average follow-up periods for those who experienced resistance transition being PZA (341.2 days), EMB (362.7 days), QUI (476.0 days), PTH (131.2 days), and INH (555.0 days), as shown in **Figure 4**. INH and RFP drug resistance at the time of initial referral was observed in 29 patients (17.5%), with the drugs with the most extended average follow-up periods for those who experienced resistance transition being PZA (341.3 days), EMB (362.7 days), PTH (131.2 days), QUI (382.0 days), and PAS (628.5 days), as shown in **Figure 5**. Among the R group, MDR-TB was observed in 29 patients (17.5%), and the annual distribution of those showing resistance to INH, RFP, and INH + RFP at the time of initial referral with resistance transition to one or more anti-TB drugs is shown in **Figure 6**.

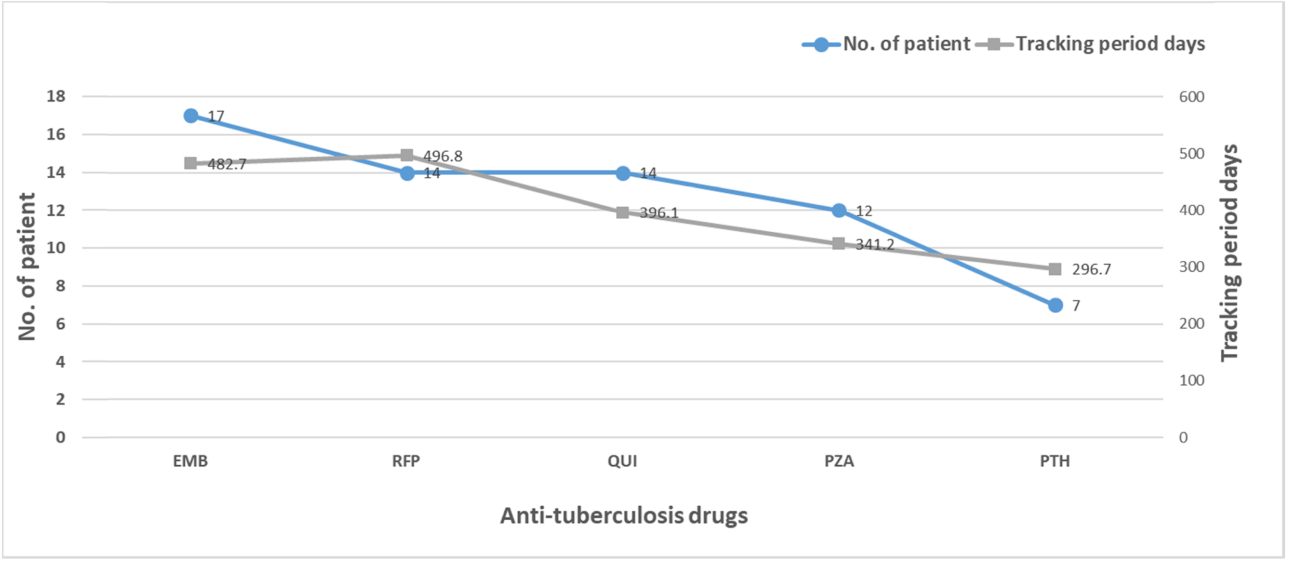

Figure 3. Pattern and average tracking period days of acquiring anti-mycobacterial resistance in R groups (cases of resistance to isoniazid anti-TB drug at the time of initial referral). Abbreviation: See **Figure 2**.

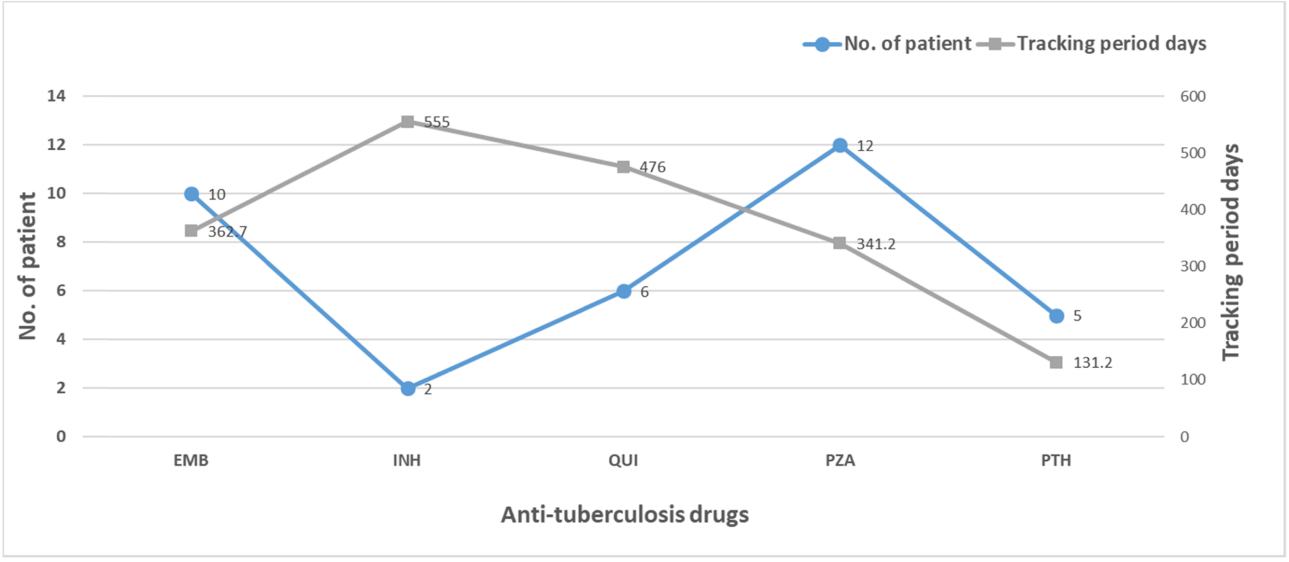

Figure 4. Pattern and average tracking period days of acquiring anti-mycobacterial resistance in R groups (cases of resistance to rifampin anti-TB drug at the time of initial referral). Abbreviation: See **Figure 2**.

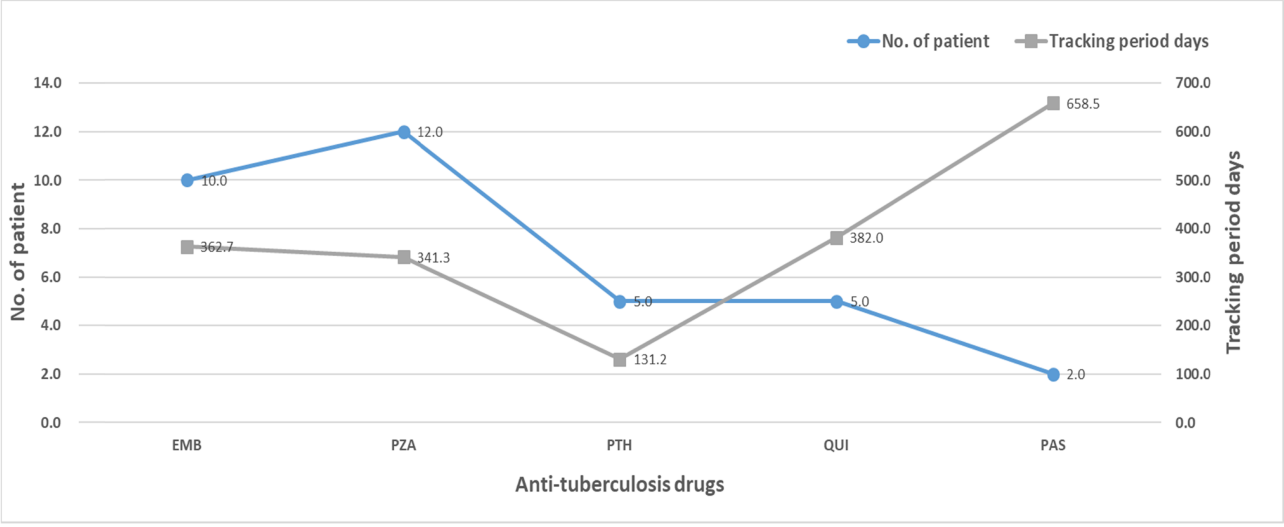

Figure 5. Pattern and average tracking period days of acquiring anti-mycobacterial resistance in R groups (cases of resistance to isoniazid and rifampin anti-TB drugs at the time of initial referral). Abbreviation: See **Figure 2**.

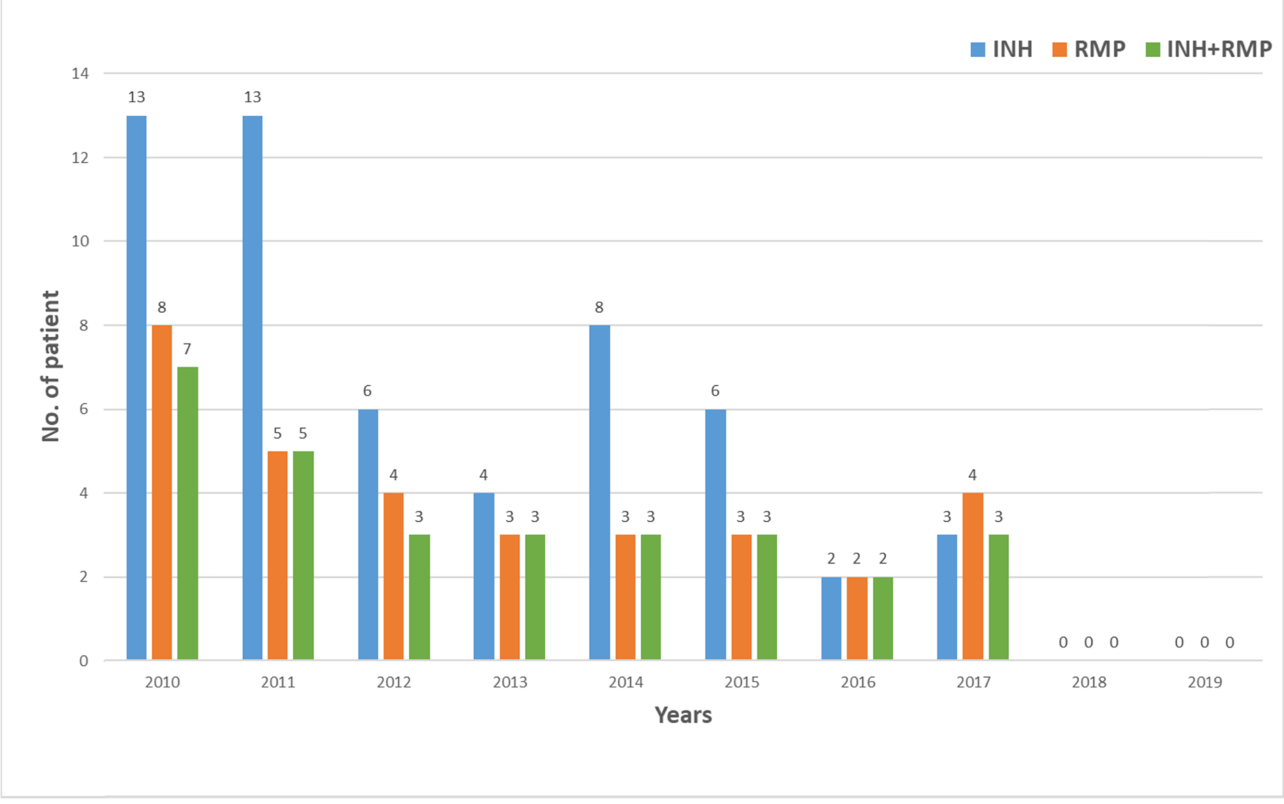

Figure 6. Cases of resistance to INH, RFP, and INH + RFP anti-TB drugs at the time of initial referral, transition of resistance to one or more anti-TB drugs. Abbreviation: See **Figure 2**.

3.3. Frequency and average follow-up period of drug resistance transition in the S group

Within the S group, 56 patients (9.4%) exhibited drug resistance to one or more anti-TB drugs, with 33 patients (5.6%) having resistance to a single drug. The results of drug resistance and the average follow-up period for the S group were as follows: AMK 2 cases (0.3%), 1,074.0 days; INH 20 cases (3.4%), 293.6 days; QUI 2 cases (0.3%), 810.0 days; RFP 6 cases (1.0%), 351.0 days; and SM 3 cases (0.5%), 266.7 days, showing resistance transition. Among the S group patients, 14 patients (2.4%) exhibited resistance transition to 2 drugs, with 10 cases (1.7%) having resistance transition to INH and RFP with 591.9 days of follow-up and 4 cases (0.7%) having resistance transition to INH and SM with 866.3 days of follow-up. There were 16 patients (2.6%) in the S group who exhibited resistance transition to 3 drugs, with 8 transitions to INH, RFP, and AMK (1.3%) with 483.0 days, 1 transition to INH, RFP, and EMB (0.2%) with 330.0 days, 1 transition to INH, RFP, and QUI (0.2%) with 504.0 days, 2 transitions to INH, RFP, and SM (0.3%) with 373.0 days, 1 transition to INH, SM, and PAS (0.2%) with 818.0 days, and 1 transition to INH, PTH, and QUI (0.2%) with 154.0 days. There was one patient (0.2%) who exhibited simultaneous resistance transition to five anti-TB drugs, including INH, RFP, SM, EMB, and PZA, with a follow-up period of 108.0 days (**Figure 7**).

INH drug resistance had the highest frequency, with 43 cases (7.2%) within the S group, and the follow-up period ranged from 98 to 1,862 days, with an average of 435.6 days. The majority of cases with resistance transition to two or more anti-TB drugs included INH. Among cases with resistance to INH, 20 patients (35.7%) exhibited resistance to INH alone with an average follow-up period of 293.6 days, 10 patients (17.9%) exhibited resistance to both INH and RFP, with an average follow-up period of 591.9 days, and 4 patients (7.1%) exhibited resistance to both INH and SM, with an average follow-up period of 866.3 days. Cases with simultaneous resistance to INH and three or more anti-TB drugs included RFP in 7 patients (12.5%) with an average follow-up period of 357.3 days, SM in 4 patients (0.7%) with an average follow-up period of 418.0 days, and EMB in 3 patients (0.5%) with an

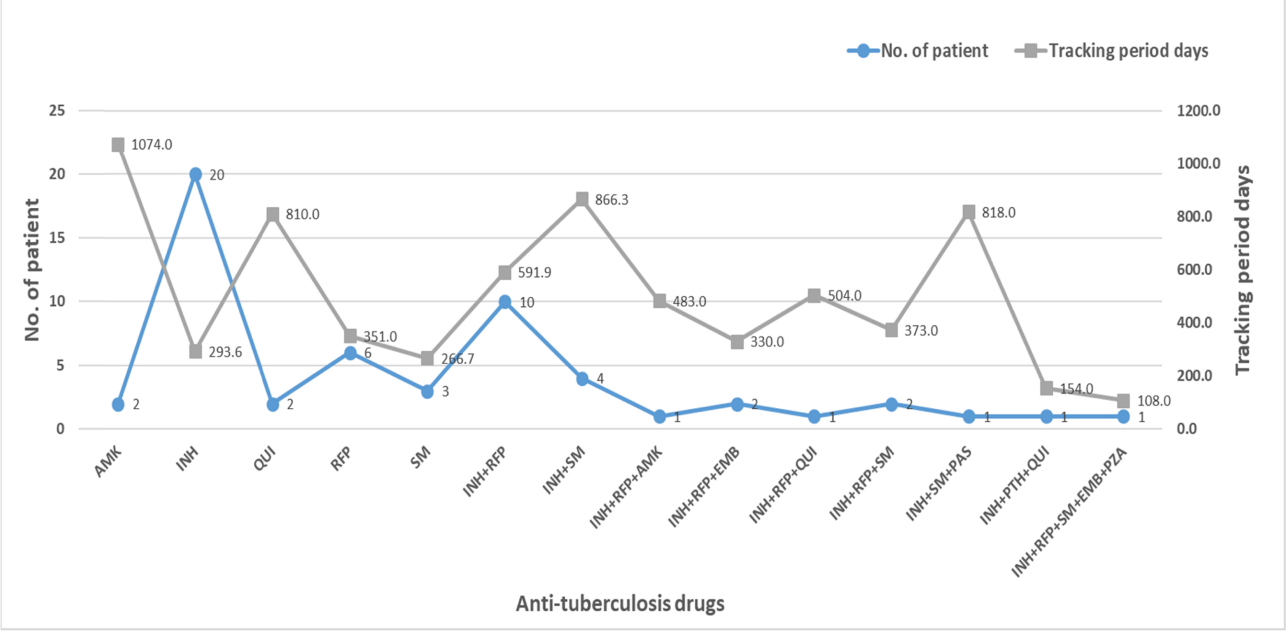

Figure 7. Pattern and average tracking period days of acquiring anti-mycobacterial resistance in S groups (cases of susceptibility to all anti-TB drugs). Abbreviations: See **Figure 2**.

average follow-up period of 256.0 days [10].

Following INH, the next most frequent drug exhibiting resistance transition was RFP, with 6 patients (10.7%) exhibiting resistance to RFP alone, with an average follow-up period of 351.0 days. For cases with resistance transition to RFP and two anti-TB drugs simultaneously, INH was involved in all of them, with 10 patients (17.9%) exhibiting resistance to both RFP and INH, with an average follow-up period of 591.9 days. Cases with simultaneous resistance to RFP and three or more anti-TB drugs included INH in all 7 patients (12.5%), with an average follow-up period of 357.3 days. There were 3 patients (0.5%) in each category of cases with simultaneous resistance to RFP and both SM and EMB.

The drug resistance transition rates for various anti-TB drugs in the S group were as follows: INH 43 cases (7.2%), RFP 23 cases (3.9%), SM 11 cases (1.9%), QUI 4 cases (0.7%), AMK 3 cases (0.5%), and EMB 3 cases (0.5%). The average follow-up periods were as follows: INH 435.6 days, RFP 157.7 days, SM 539.7 days, QUI 569.5 days, AMK 877.0 days, and EMB 253.0 days. Furthermore, cases with resistance transition to RFP drugs involved INH in all cases, while cases with resistance transition to INH drugs did not (**Figure 8**). The S group had 17 patients (2.9%) who transitioned to MDR-TB, with all of them being susceptible to all anti-TB drugs at the time of the initial referral. Among these individuals, resistance transition to INH, RFP, and INH+RFP was observed, as shown in the annual distribution in **Figure 9**.

4. Discussion

Innovative research and development in diagnostics, treatment, and prevention are essential to achieve the goals of TB treatment. Currently, research is actively underway and expanding in areas such as new anti-TB drugs, treatment regimens, vaccine development, and novel diagnostic methods, including the use of cutting-edge techniques such as whole genome sequencing [11].

XDR-TB is more challenging to treat and has a higher mortality rate compared to MDR-TB. According to domestic research, the treatment success rate for 75 individuals with XDR-TB is reported at 29%, with a mortality rate of 49% [12]. The principles of treatment for XDR-TB are based on those for MDR-TB, and

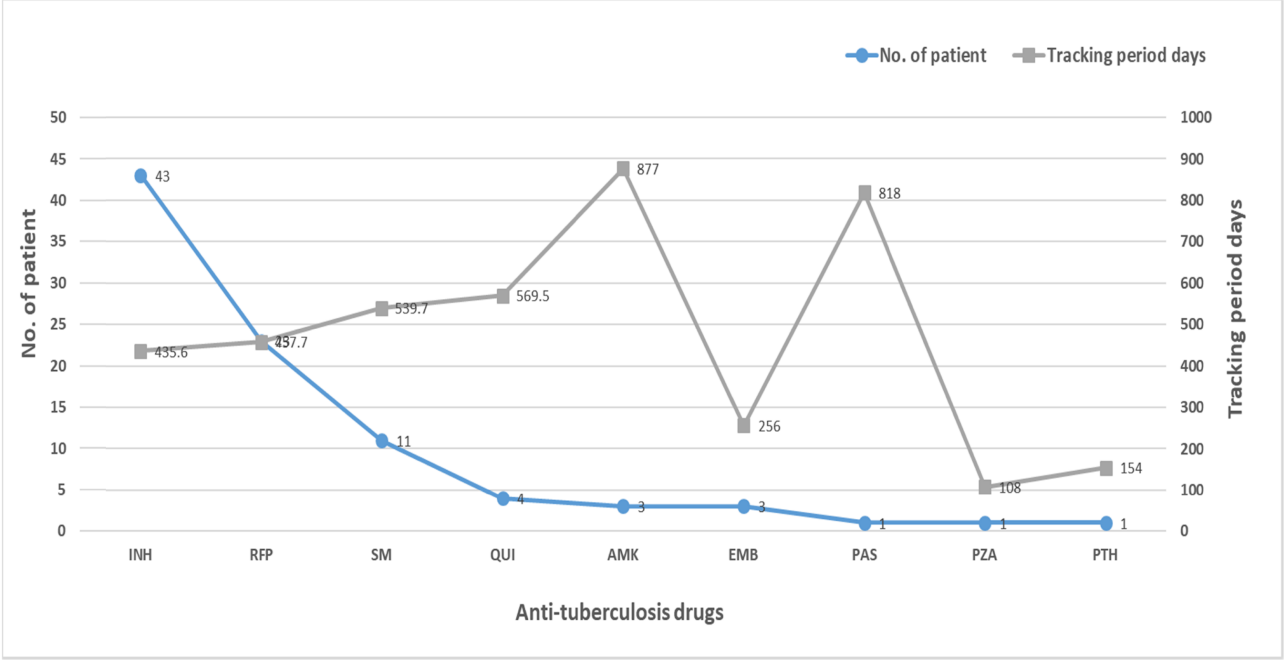

Figure 8. Frequency and average tracking period days of acquiring anti-mycobacterial resistance in S groups (cases of susceptibility to all anti-TB drugs). Abbreviations: See **Figure 2**.

the principles for drug selection are not significantly different [13,14]. However, the diverse patterns of drug resistance make it challenging to create an effective drug regimen. The treatment of both MDR-TB and XDR-TB is crucial for preventing the development of resistance and rapidly diagnosing drug resistance. Additionally, patient education and adherence support, as well as management of side effects, are essential to enhance patients' treatment compliance [15].

An analysis of MDR-TB patients in Bangladesh resulted in a short-course standard treatment for MDR-TB, consisting of kanamycin, gatifloxacin, prothionamide, clofazimine, ethambutol, pyrazinamide, and isoniazid for the initial four months, followed by ethambutol, pyrazinamide, and clofazimine for five months. This regimen achieved a high treatment success rate of 82.5%, a low treatment discontinuation rate of 5.8%, and a treatment failure rate of 0.5% in 206 patients [16]. According to reports in South Korea, the frequency of drug resistance in samples confirmed for drug-resistant TB showed the following order for specific drugs: INH 29.7%, RFP 21.6%, EMB 15.5%, SM 13.0%, PAS 11.8%, OFX11.1%, PZA 10.6%, ETH 8.5%, KM 6.6%, and CS 6.5%. It was reported that the majority of cases (66.5%) exhibited resistance to one to four drugs [17]. The treatment success rate for patients diagnosed with MDR-TB in 2015 was 54%, while the treatment success rate for XDR-TB patients remained at 34%. Among patients who initiated treatment, 8% failed, 15% died during treatment, and 14% discontinued treatment. One of the main reasons for these outcomes is the current treatment regimen's lack of efficacy, significant side effects, and the need for extended treatment periods [18].

Among the cases with resistance to at least one anti-TB drug, it was observed that resistance to INH was the highest, with 55 cases (33.1%) showing resistance at the initial referral, and drug resistance transition to EMB with 17 cases (26.6%), RFP with 14 cases (21.9%), QUI with 14 cases (21.9%), and

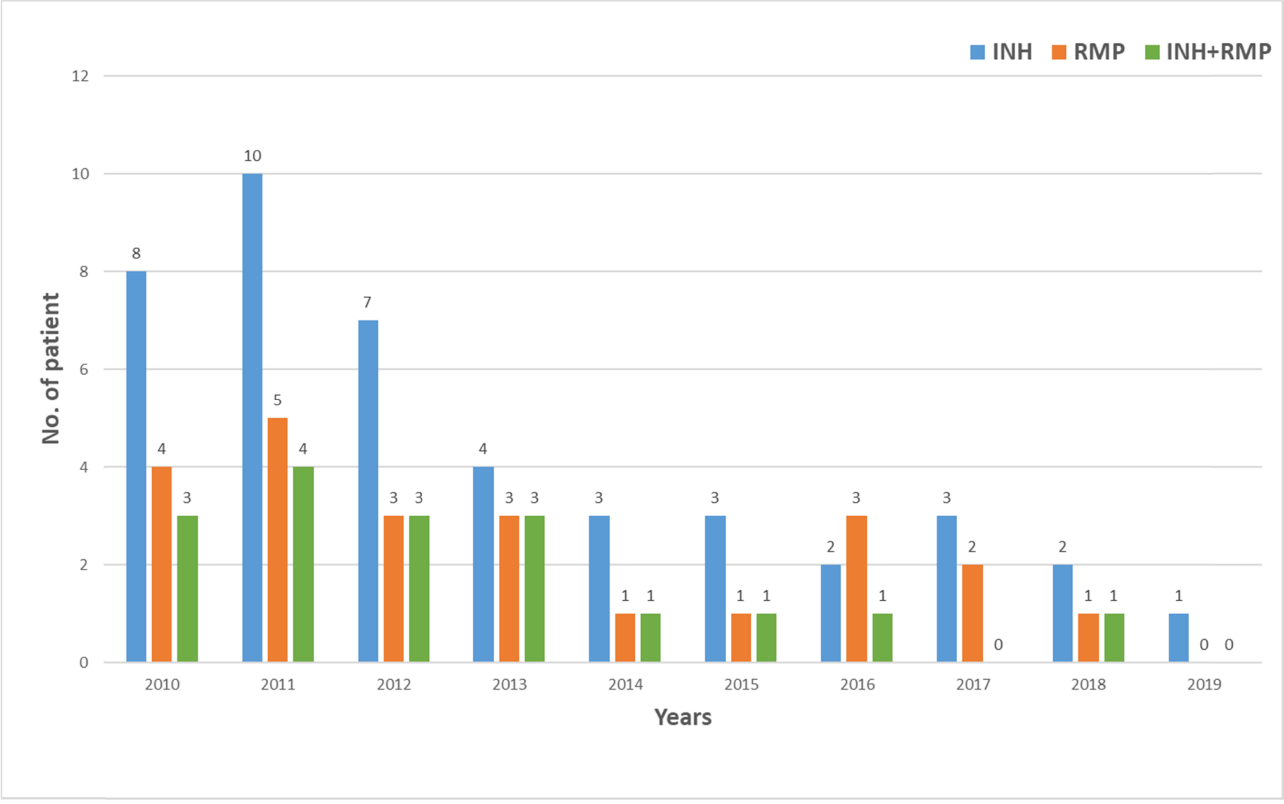

Figure 7. Cases of susceptibility to all anti-TB drugs at the time of initial referral, transition of resistance to INH, RFP, and INH + RFP anti-TB drugs. Abbreviation: See **Figure 2**.

PZA with 12 cases (10.9%). Additionally, resistance to RFP at the initial referral was observed in 32 patients (19.3%), and drug resistance transition to PZA with 12 cases (34.3%), EMB with 10 cases (28.6%), QUI with 6 cases (17.1%), PTH with 5 cases (14.3%), and INH with 2 cases (5.7%). Cases that showed resistance to both INH and RFP at the initial referral had a total of 29 cases (17.5%), and drug resistance transition to PZA with 12 cases (35.3%), EMB with 10 cases (29.4%), PTH with 5 cases (14.7%), QUI with 5 cases (14.7%), and PAS with 2 cases (5.9%). In cases with susceptibility to all 10 anti-TB drugs, the highest frequency of resistance development was observed in INH, with 43 cases (7.2%). The average tracking period for these cases was 435.6 days. The rate of resistance development for other anti-TB drugs, among cases developing resistance, was as follows: RFP 3.9%, SM 1.9%, QUI 0.7%, AMK 0.5%, and EMB 0.5%. The average tracking periods for resistance development were as follows: RFP 157.7 days, SM 539.7 days, QUI 569.5 days, AMK 877.0 days, and EMB 253.0 days.

The transition from drug susceptibility to resistance, particularly in patients transitioning from MDR-TB to XDR-TB, provides valuable insights. These findings can be highly informative for TB treatment, assisting in patient care.

Disclosure statement

The authors declare no conflict of interest.

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