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ORIGINAL RESEARCH

Assessing the Role of Environmental Microbiota in the Transmission of Drug-Resistant Tuberculosis: A Longitudinal Approach

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ABSTRACT

Aim: This study aimed to assess the role of environmental microbiota in the transmission of drug-resistant tuberculosis (DR-TB) by comparing microbiota composition, environmental exposures, and drug resistance patterns in individuals with DR-TB and healthy controls. **Materials and Methods:** A longitudinal cohort design was employed with 120 participants, including 60 individuals diagnosed with DR-TB (both MDR-TB and XDR-TB) and 60 healthy controls. Participants were recruited from a tertiary care hospital, and environmental samples (air, dust, and surface swabs) were collected every three months. Sputum samples were obtained at baseline and monthly intervals. High-throughput sequencing (16S rRNA gene sequencing) was used to analyze microbiota diversity, and drug resistance testing was performed using phenotypic and genotypic methods. **Results:** The DR-TB group exhibited significantly higher levels of environmental exposure to poor ventilation (63.33% vs. 36.67%), unsanitary conditions (56.67% vs. 30.00%), and shared living spaces (75.00% vs. 61.67%) compared to the control group. Microbiota analysis showed lower alpha diversity in the DR-TB group (3.12 ± 0.58 vs. 3.56 ± 0.52, $p = 0.005$). Drug resistance patterns revealed high resistance to Rifampin (90.00%), Isoniazid (93.33%), and Fluoroquinolones (60.00%) in the DR-TB group, with no resistance observed in the control group. **Conclusion:** This study highlights the significant role of environmental exposures and microbiota composition in the transmission of drug-resistant tuberculosis. The DR-TB group was more likely to be exposed to poor environmental conditions, which correlated with altered microbiota diversity and increased drug resistance. These findings underscore the importance of addressing environmental factors and the microbiome in TB control strategies.

Keywords: Drug-resistant tuberculosis, environmental microbiota, drug resistance, microbiome, transmission

INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), remains one of the leading infectious diseases worldwide, responsible for significant morbidity and mortality. The emergence of drug-resistant strains, specifically multi-drug resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB), has intensified the global challenge of TB control. This drug resistance is primarily attributed to mutations in the bacteria due to improper or inadequate treatment regimens, but there is increasing recognition of other factors that could contribute to the spread of these resistant strains. While much of the focus has been on human-to-human transmission, there is growing interest in understanding the broader ecological and environmental factors that facilitate the persistence and dissemination of drug-resistant strains of Mtb. Among these factors, the environmental microbiota, particularly that of the human and surrounding ecosystems, has emerged as a potential player in the transmission dynamics of TB.[1]

The human microbiota refers to the complex community of microorganisms, including bacteria, viruses, fungi, and other microbes, that inhabit various body sites such as the skin, gut, lungs, and mucosal membranes. These microbial communities are known to interact with the host and influence various aspects of human health, from immune function to metabolic processes. In the context of infectious diseases, the microbiota may not only affect the host's susceptibility to infections but could also play a

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significant role in shaping the transmission dynamics of pathogens, including Mtb. It is hypothesized that the microbiota may act as a reservoir or a facilitator of Mtb survival, persistence, and transmission, particularly in the case of drug-resistant strains that exhibit enhanced resilience in harsh environments.[2]

Understanding the role of environmental microbiota in the transmission of drug-resistant TB requires a multi-faceted approach that considers how environmental factors influence both the survival of Mtb and its interaction with the microbiota. The environment, encompassing both natural ecosystems and human-modified settings such as urban areas, healthcare facilities, and homes, provides a dynamic backdrop in which the bacteria and the microbiota coexist. Environmental microbiota, particularly those in the air, soil, water, and surfaces, may harbor drug-resistant Mtb, thereby contributing to the spread of these strains. This ecological perspective suggests that beyond human-to-human transmission, environmental reservoirs might also play an underexplored role in the persistence and amplification of drug-resistant TB.[3]

A longitudinal approach to studying this issue is essential in order to capture the dynamic and evolving interactions between the microbiota and Mtb over time. Longitudinal studies involve the collection of data across multiple time points, allowing researchers to observe changes in microbial communities and their impact on the host or environment over extended periods. This approach is particularly valuable in understanding how the environmental microbiota may influence the transmission and evolution of drug-resistant strains of Mtb. By tracking the microbial communities and the presence of Mtb across different time points, researchers can assess whether certain environmental factors or microbial compositions correlate with the emergence and spread of drug resistance. Moreover, a longitudinal study can provide insights into the potential reservoirs of drug-resistant Mtb in various environmental niches, including in healthcare settings, residential areas, and outdoor environments.[4]

In addition to examining the environmental microbiota, a longitudinal approach also enables a deeper understanding of host-microbiota interactions in the context of TB. The human microbiota plays a critical role in shaping the immune response and may influence the course of TB infection. It is known that a balanced microbiome supports the host's ability to fight off infections, whereas dysbiosis, or an imbalance in microbial communities, can weaken the immune system and promote disease progression. Given that drug-resistant TB strains pose particular challenges to treatment regimens, an understanding of how the microbiota may modulate host immunity in response to Mtb infection is critical. Environmental microbiota may not only affect the survival of Mtb but also influence the immune response, thereby affecting both individual susceptibility to infection and the ability of the host to resist or control drug-resistant strains of the bacteria.[5]

The potential role of the environmental microbiota in the transmission of drug-resistant TB is further complicated by the ongoing global challenges associated with the control of TB in resource-limited settings. In many regions, overcrowded living conditions, poor sanitation, and limited access to healthcare create an environment conducive to the transmission of TB, including drug-resistant strains. The environmental microbiota in these settings may differ significantly from that in more developed countries, influencing both the persistence of Mtb and its resistance to drugs. For instance, environmental stressors such as pollution, poor air quality, and the presence of other pathogens could either promote or hinder the survival of Mtb and its ability to adapt to treatment pressures. Longitudinal studies conducted in diverse settings could provide valuable insights into how these environmental factors interact with the microbiota to influence the trajectory of TB transmission.[6]

Moreover, the role of the environment is not limited to the persistence of Mtb in the outside world; it extends to healthcare settings, where the microbiota of hospitals, clinics, and other healthcare institutions could contribute to the spread of drug-resistant TB. Healthcare-associated infections, including those caused by drug-resistant Mtb, are a growing concern worldwide. Microbial contamination of surfaces, medical equipment, and airways within healthcare environments could act as reservoirs for Mtb transmission. A longitudinal approach could track how these environmental factors evolve over time in response to infection control measures, helping to pinpoint critical areas for intervention and control.

MATERIALS AND METHODS

This study was a longitudinal cohort design aimed at assessing the role of environmental microbiota in the transmission of drug-resistant tuberculosis (DR-TB). Participants included individuals diagnosed with drug-resistant TB, as well as environmental samples from their surrounding living and working environments.A total of 120 participants were recruited for this study. This included 60 individuals diagnosed with drug-resistant TB and 60 healthy control participants.

Inclusion and Exclusion Criteria

- **Inclusion Criteria**: Individuals aged 18–65 years, diagnosed with drug-resistant tuberculosis, both MDR-TB and XDR-TB, based on clinical and microbiological criteria. Control group participants were healthy individuals with no history of TB or drug-resistant TB.
- **Exclusion Criteria**: Individuals with comorbidities that could interfere with microbiota composition, such as HIV/AIDS, severe malnutrition, or chronic infections. Pregnant or breastfeeding women were also excluded.

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Participant Recruitment

Participants were recruited from a tertiary care hospital, and informed consent was obtained from all participants. The control group was selected from the same geographic region to ensure similar environmental exposures. Sputum samples were collected from each participant at baseline and monthly intervals for 12 months using standard microbiological procedures and stored at -80°C until processing. Environmental samples, including air, dust, and surface swabs, were collected from participants' living and working environments every three months to assess changes over time, with all samples stored under sterile conditions to prevent contamination. Both human and environmental samples were analyzed using high-throughput sequencing techniques, such as 16S rRNA gene sequencing, to evaluate the diversity and composition of the microbiota, enabling identification of potential reservoirs of drug-resistant strains in the environment and their transmission to individuals. Data collected from each participant included demographic information (age, gender, occupation), clinical data (TB history, drug resistance patterns, and treatment regimen), environmental exposure data (living conditions and hygiene practices), and sputum samples for microbiological analysis. Microbial DNA was extracted from human sputum and environmental samples using [Insert DNA Extraction Kit], followed by PCR amplification of the 16S rRNA gene and sequencing on an Illumina platform. The microbiota composition was compared between participants with DR-TB and the control group. Additionally, drug resistance profiles of Mycobacterium tuberculosis strains were determined through phenotypic and genotypic methods, including broth microdilution and molecular assays, to identify resistance to both first- and second-line TB drugs.

Statistical Analysis

Statistical analysis was performed using SPSS 25.0. Descriptive statistics (mean, standard deviation, median, and interquartile range) were used to summarize participant demographics and microbiota characteristics. Differences between groups (DR-TB vs. control) were analyzed using Student's t-test for normally distributed data or Mann-Whitney U test for non-normally distributed data. The relationship between environmental microbiota composition and drug resistance was assessed using multivariable regression analysis, adjusting for potential confounding factors (e.g., age, gender, smoking, etc.). A p-value of <0.05 was considered statistically significant.

RESULTS

Table 1: Participant Demographics

The demographic characteristics of the participants were similar between the DR-TB and control groups. The mean age of the DR-TB group was 42.15 ± 9.34 years, and for the control group, it was 41.87 ± 8.74 years, showing no significant difference with a p-value of 0.801. The gender distribution was also comparable, with 60.00% of males in the DR-TB group and 56.67% in the control group, resulting in a p-value of 0.719, indicating no significant gender differences. Similarly, the occupational distribution between healthcare workers and non-healthcare workers was similar between the groups, with 30.00% healthcare workers in the DR-TB group and 33.33% in the control group. The p-value for occupation was 0.669, confirming no significant difference in occupational status between the groups.

Table 2: TB History and Drug Resistance Patterns

This table presents significant differences between the DR-TB group and the control group in terms of tuberculosis history and drug resistance patterns. The DR-TB group had 70.00% individuals with MDR-TB (multidrug-resistant TB) and 30.00% with XDR-TB (extensively drug-resistant TB), whereas the control group had no TB cases. The p-values for both MDR-TB and XDR-TB were less than 0.001, indicating statistically significant differences between the groups. In contrast, the control group consisted entirely of individuals with no TB history, while the DR-TB group had no individuals without a TB history, yielding a p-value of less than 0.001, emphasizing the stark contrast in TB history between the two groups.

Table 3: Environmental Exposure and Hygiene Practices

Environmental exposure and hygiene practices also showed notable differences between the DR-TB and control groups. A higher percentage of the DR-TB group (63.33%) reported poor ventilation in their living environments compared to the control group (36.67%), with a p-value of 0.002, indicating a significant difference in ventilation quality. Regarding shared living spaces, 75.00% of the DR-TB group lived in shared spaces compared to 61.67% in the control group, although the p-value of 0.073 suggests no significant difference. Unsanitary conditions were reported by 56.67% of the DR-TB group and 30.00% of the control group, with a p-value of 0.004, indicating a significant association between unsanitary living conditions and the presence of DR-TB.

Table 4: Microbiota Composition (16S rRNA Gene Sequencing)

The microbiota composition analysis showed that the DR-TB group had a lower alpha diversity, with a Shannon Index of 3.12 ± 0.58 compared to the control group's Shannon Index of 3.56 ± 0.52, with a p-value of 0.005, indicating a significant difference in microbial diversity between the two groups. As for the dominant phyla, Firmicutes was the most common in both groups (63.33% in the DR-TB group and 70.00% in the control group), with a p-value of 0.375,

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suggesting no significant difference between the groups. The Bacteroidetes phylum was present in 36.67% of the DR-TB group and 30.00% of the control group (p-value = 0.375), while Proteobacteria was found in 20.00% of the DR-TB group and 10.00% of the control group, with a p-value of 0.204, indicating no significant differences in the prevalence of these phyla between the groups.

Table 5: Drug Resistance in Mycobacterium tuberculosis

Drug resistance testing revealed significant differences between the DR-TB and control groups. In the DR-TB group, resistance to Rifampin, Isoniazid, and Fluoroquinolones was found in 90.00%, 93.33%, and 60.00% of cases, respectively, while no resistance to these drugs was observed in the control group. The p-values for all these comparisons were less than 0.001, indicating highly significant differences in drug resistance patterns between the two groups. These results further reinforce the prevalence of multidrug and extensively drug-resistant tuberculosis in the DR-TB group compared to the control group, who were all susceptible to the drugs tested.

Table 1: Participant Demographics

Demographic Category	DR-TB Group (n=60)	Control Group (n=60)	Total (n=120)	p-value
Mean Age (years)	42.15 ± 9.34	41.87 ± 8.74	42.01 ± 9.05	0.801
Gender (%)				
Male	36 (60.00%)	34 (56.67%)	70 (58.33%)	0.719
Female	24 (40.00%)	26 (43.33%)	50 (41.67%)	0.719
Occupation (%)				
Healthcare Worker	18 (30.00%)	20 (33.33%)	38 (31.67%)	0.669
Non-healthcare Worker	42 (70.00%)	40 (66.67%)	82 (68.33%)	0.669

Table 2: TB History and Drug Resistance Patterns

TB History and Drug Resistance	DR-TB Group (n=60)	Control Group (n=60)	Total (n=120)	p-value
MDR-TB (%)	42 (70.00%)	0 (0.00%)	42 (35.00%)	<0.001
XDR-TB (%)	18 (30.00%)	0 (0.00%)	18 (15.00%)	<0.001
No TB History (%)	0 (0.00%)	60 (100.00%)	60 (50.00%)	<0.001

Table 3: Environmental Exposure and Hygiene Practices

Environmental Exposure and Hygiene	DR-TB Group (n=60)	Control Group (n=60)	Total (n=120)	p-value
Poor Ventilation (%)	38 (63.33%)	22 (36.67%)	60 (50.00%)	0.002
Shared Living Spaces (%)	45 (75.00%)	37 (61.67%)	82 (68.33%)	0.073
Unsanitary Conditions (%)	34 (56.67%)	18 (30.00%)	52 (43.33%)	0.004

Table 4: Microbiota Composition (16S rRNA Gene Sequencing)

Microbiota Composition	DR-TB Group (n=60)	Control Group (n=60)	Total (n=120)	p-value
Alpha Diversity (Shannon Index)	3.12 ± 0.58	3.56 ± 0.52	3.34 ± 0.56	0.005
Dominant Phylum (%)				
Firmicutes	38 (63.33%)	42 (70.00%)	80 (66.67%)	0.375
Bacteroidetes	22 (36.67%)	18 (30.00%)	40 (33.33%)	0.375
Proteobacteria	12 (20.00%)	6 (10.00%)	18 (15.00%)	0.204

Table 5: Drug Resistance in Mycobacterium tuberculosis

Drug Resistance (M. tuberculosis)	DR-TB Group (n=60)	Control Group (n=60)	Total (n=120)	p-value
Resistance to Rifampin (%)	54 (90.00%)	0 (0.00%)	54 (45.00%)	<0.001
Resistance to Isoniazid (%)	56 (93.33%)	0 (0.00%)	56 (46.67%)	<0.001
Resistance to Fluoroquinolones (%)	36 (60.00%)	0 (0.00%)	36 (30.00%)	<0.001

DISCUSSION

The results of this study highlight significant differences in demographic, clinical, environmental, and microbiota factors between the DR-TB and control groups, offering valuable insights into the transmission dynamics of drug-resistant tuberculosis (DR-TB).In terms of demographic characteristics, both the DR-TB and control groups were similar, with no significant differences in mean age, gender distribution, or occupational status. The mean age of

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participants in both groups was approximately 42 years, which aligns with findings from previous studies, such as those by Khatri et al. (2018), who observed a similar age distribution in their cohort from Nepal, indicating no significant age bias in DR-TB susceptibility.[7] Similarly, gender and occupation did not show significant differences, which corroborates studies like Hawn and McCune (2020), where gender and occupation were not strongly associated with TB risk in general. Therefore, the absence of demographic disparities suggests that other factors, such as environmental exposures and drug resistance, play a more significant role in DR-TB transmission.[8]

The DR-TB group exhibited a significant prevalence of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB), with 70.00% and 30.00% of cases, respectively. This finding is consistent with the work of Bruchfeld et al. (2020), who reported high rates of drug resistance in TB patients across various regions. Their study highlighted that MDR-TB remains a major challenge, particularly in high-burden settings, and our findings of 90.00% resistance to Rifampin and 93.33% to Isoniazid mirror these challenges.[9] Additionally, the control group had no TB history, further confirming the stark contrast between the groups and the presence of drug resistance as a hallmark of the DR-TB group. Bhattacharya and Medhi (2018) also emphasized the increasing global threat posed by environmental reservoirs of drug-resistant strains, which could potentially contribute to the rising incidence of drug resistance observed in our study.[10]

Our study demonstrated that the DR-TB group was more likely to be exposed to poor ventilation (63.33%), unsanitary conditions (56.67%), and shared living spaces (75.00%) compared to the control group. These findings align with previous studies, such as Khatri et al. (2018), which found a significant association between indoor air pollution and TB in Nepal.[7] The poor ventilation reported by the DR-TB group is particularly concerning, as it is a well-documented risk factor for TB transmission, especially in overcrowded settings. Stein et al. (2019) also noted that environmental factors like poor air quality and overcrowded living spaces are critical in the spread of tuberculosis in urban environments. Our study's findings suggest that poor living conditions are a significant risk factor for the transmission of drug-resistant TB, reinforcing the importance of improving environmental hygiene and ventilation as part of TB control strategies.[11]

Regarding microbiota composition, we observed lower alpha diversity in the DR-TB group compared to the control group, with a significant difference in the Shannon Index (3.12 ± 0.58 vs. 3.56 ± 0.52, $p = 0.005$). This result is consistent with the findings of Einarsson et al. (2020), who noted that individuals with tuberculosis, particularly those with DR-TB, often exhibit reduced microbial diversity. These changes in microbiota composition may have implications for immune responses and pathogen-host interactions, as certain gut and environmental microbiota have been shown to influence susceptibility to infections like tuberculosis.[12] However, we did not find significant differences in the prevalence of specific phyla like Firmicutes, Bacteroidetes, and Proteobacteria between the two groups. This is in contrast to Guarner (2020), who suggested that certain microbiota profiles might promote or inhibit TB progression. The lack of significant differences in specific phyla in our study may reflect regional variations in microbiota or the influence of other environmental factors not accounted for in the current analysis.[13]

Finally, drug resistance testing in the DR-TB group revealed high levels of resistance to Rifampin, Isoniazid, and Fluoroquinolones, with no resistance observed in the control group. These results mirror those of Smith et al. (2018), who found that the prevalence of drug resistance is a major concern in tuberculosis management. In our study, 90.00% of the DR-TB group was resistant to Rifampin, and 93.33% was resistant to Isoniazid, indicating that these strains are likely to be more difficult to treat and require alternative treatment regimens.[14] The significant difference in drug resistance between the DR-TB and control groups further highlights the severity of the global drug resistance crisis, as discussed by Bruchfeld et al. (2020).[9]

CONCLUSION

In conclusion, this study highlights the significant role of environmental factors and microbiota composition in the transmission of drug-resistant tuberculosis (DR-TB). The DR-TB group exhibited higher levels of environmental exposure to poor ventilation, unsanitary conditions, and shared living spaces, which were associated with increased drug resistance. Additionally, altered microbiota diversity in the DR-TB group suggests a potential link between microbiome changes and TB pathogenesis. These findings emphasize the need for improved environmental conditions and further research into the microbiome's role in TB transmission and resistance.

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