TOPIC IN REVIEW

How molecular epidemiology has changed what we know about tuberculosis

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Summary points

- A variety of social and biologic factors foster the accelerated progression and transmission of tuberculosis
- Many populations have recently had high rates of tuberculosis transmission
- Transmission may disproportionately occur among identifiable subgroups and in specific locations
- Patients with smears that are negative for acid-fast bacilli transmit infection to others, although they are less infectious than patients with smears positive for the bacilli
- Continued vigilance is needed to prevent and identify false-positive cultures due to laboratory cross-contamination
- People may become exogenously reinfected with *Mycobacterium tuberculosis*, and this can be clinically indistinguishable from relapsed disease
- Aggressive implementation of currently available control measures can decrease transmission

By the mid-1980s, the US public health community considered tuberculosis to be under control, and a plan was established for its elimination by 2010. Between 1989 and 1992, however, the number of cases increased, and in response, scientists and public health officials reinvigorated research and control programs. These efforts have now turned the tide, and the number of cases of tuberculosis in the United States is again decreasing, being down 31% from the peak of the resurgence.[1]

During this time, researchers and disease controllers came together in a relatively new field, molecular epidemiology. This endeavor combines molecular methods for identifying individual strains of bacteria with conventional epidemiologic methods to investigate the determinants and distribution of disease. Together they can establish transmission links, identify risk factors for transmission, and provide insight into the pathogenesis of tuberculosis. Bacterial DNA fingerprinting is performed using restriction fragment-length polymorphism analysis that yields a unique pattern for unrelated clinical organisms and an identical pattern for strains isolated in outbreak settings. It can, however, be used only to track transmission between persons with active disease because the technique requires a viable culture of *Mycobacterium tuberculosis*.[2]

In this review, we present a summary of what has been learned about transmission dynamics and the pathogenesis of *M tuberculosis* since the first description of DNA fingerprinting in 1989 and the implication of these lessons for tuberculosis control.

TUBERCULOSIS CAN PROGRESS RAPIDLY FROM INFECTION TO DISEASE

The natural history of tuberculosis starts with the exposure to *M tuberculosis*. Although the human immune response generally controls this infection, it cannot eradicate the pathogen, resulting in an asymptomatic infection. At this point, a person is noninfectious, and the only evidence of infection is reactivity to a tuberculin skin test. It has been widely stated that active tuberculosis will develop in only 10% of these latently infected people during their lifetimes. The ability of bacterial DNA fingerprinting to precisely track *M tuberculosis* shows that in certain circumstances, this sequence of events is "telescoped," with the interval from infection to disease being as short as a few weeks.

In general, if people have been infected from each other or a common source, their strains will have the same "fingerprint." Conversely, if the persons have reactivated latent infection, their fingerprints will differ. Using this approach, it has been shown that persons infected with the human immunodeficiency virus (HIV),[3] homeless persons,[4] and other immunosuppressed patients[5] developed active tuberculosis within 4 weeks to 6 months of being exposed to *M tuberculosis*. These data confirm that host factors like immunosuppression can change the natural history of disease and emphasize the need to rapidly identify these people so that infection may be diagnosed and treated. Also, the comparison of DNA fingerprinting of *M tuberculosis* in outbreaks has become a routine tool for

Glossary

Molecular epidemiology of tuberculosis A field that combines molecular techniques and conventional epidemiologic methods to study the dynamics of tuberculosis transmission

Tuberculosis DNA fingerprinting The use of molecular biologic techniques to identify specific strains of bacteria

Restriction fragment-length polymorphism analysis A technique used to fingerprint *Mycobacterium tuberculosis*

investigating cases in which tuberculosis transmission is suspected.

A DISTURBING PERCENTAGE OF ADULT CASES OF TUBERCULOSIS ARE RECENTLY ACQUIRED

Before the resurgence of tuberculosis, it was thought that tuberculosis develops in most adults by the reactivation of infection acquired in the remote past. By extrapolating the ability of DNA fingerprinting to identify recently transmitted disease in outbreaks, it has been possible to estimate the relative contribution of reactivated and recently transmitted disease. These estimates are made by comparing the DNA fingerprint of all tuberculosis organisms in a population and identifying groups of people infected with the same strain. The assumption is that among a group of patients infected with the same strain (termed "clusters"), one represents a reactivated case, and the remainder will be due to recent infection. One person from each cluster and all persons found to have disease by unique strains are assumed to have reactivated disease.

In contrast to the established dogma, molecular epidemiologic studies have shown that about 38% of the tuberculosis cases in New York City from 1989 to 1992[6] and 31% of cases in San Francisco, California during 1991 and 1992[7] were due to recent infection. Subsequently, similar studies have found this number to vary considerably in different epidemiologic circumstances.[8,9]

These findings have important implications for tuberculosis control. If recent infection with rapid evolution to active tuberculosis is more common, efforts should focus on identifying and treating the source patients and investigating all the contacts. Conversely, in communities where transmission is rare and most disease is a consequence of reactivated latent infection, control measures must be directed to identify and treat people who are latently infected, a situation that is increasingly common in the United States.

RISK FACTORS FOR TUBERCULOSIS TRANSMISSION ARE IDENTIFIABLE

In addition to quantifying the amount of recent transmission, molecular epidemiologic studies can identify the risk factors for these events and suggest methods for interrupting transmission.

Tuberculosis has been associated with alcohol abuse, but a study of an outbreak showed that visiting a bar and not the alcohol use was the risk factor for *M tuberculosis* transmission in this setting.[10] By comparing people who are in clusters with those infected with unique strains, it is possible to define risk factors for tuberculosis transmission in a population. In San Francisco, tuberculosis patients younger than 60 years, of African American or Hispanic ethnicity, and those who were HIV-seropositive were more likely to have been recently infected with *M tuberculosis*.[7]

It was traditionally thought that prolonged contact was needed for tuberculosis transmission between an infectious patient and a susceptible host. Accordingly, contact investigation focused on household, school, and workplace contacts. Molecular epidemiologic studies, however, have shown unsuspected sites of transmission. For example, in Seattle, Washington, an abrupt increase of tuberculosis was detected among people infected with HIV. The initial contact investigation did not reveal any epidemiologic link, but the DNA fingerprints of a third of patients were similar.[10] The new investigations showed that these patients had a common exposure in a bar.

Hospitals are also important sites of tuberculosis transmission. Several outbreaks have been documented to be mainly caused by a delay of tuberculosis diagnosis in the source patient and susceptible persons, such as those infected with HIV.[11]

Control efforts must be specifically enhanced in those social scenes and locations where most transmission is occurring. In the general population, passive case finding of tuberculosis (detection among persons seeking medical attention because of chronic cough) is recommended. In some settings, however, more active searches for symptomatic persons may be needed to control the disease. In hospitals, strict respiratory isolation policies are essential, particularly where patients or staff may be infected with HIV. Conventional contact investigations identify only a small percentage of transmission, stressing the need for novel approaches.[12] What combination of these activities will be most cost-effective for interrupting transmission remains to be determined.

PATIENTS WITH SMEARS NEGATIVE FOR ACID-FAST BACILLI ARE INFECTIOUS

Given the limited budgets of tuberculosis control programs, prioritizing control activities is a challenge. One approach involves the microscopic examination of sputum specimens to detect acid-fast bacilli (AFB). Patients whose sputum specimens have microscopically demonstrable AFB are highly infectious, and control measures such as isolation are essential. Recently, transmission from patients with AFB-negative smears was examined in a population-based study. It was concluded that although patients with AFB-positive smears were three to four times more infectious than patients whose smears were AFB-negative, 17% of cases of tuberculosis transmission originated from people with AFB-negative smears.[13] A study in rural Mexico showed that primary drug-resistant tuberculosis (resistant organisms in patients without a history of antituberculosis treatment) was more frequent in patients whose smears were AFB-negative than those whose smears were AFB-positive.[14] Taken together, these studies suggest

that smear-negative patients transmit a considerable amount of infection and may even disproportionately propagate drug-resistant *M tuberculosis*.

These findings may have grave implications for tuberculosis control programs in developing countries, where diagnosis is based on AFB smears. It is important to expand the use of existing technologies to identify patients with tuberculosis who have AFB-negative smears.

PATIENTS WHOSE TUBERCULOSIS WAS CURED MAY HAVE SUBSEQUENT EPISODES

Whether tuberculosis confers protective immunity or patients may become reinfected with *M tuberculosis* has long been debated. To address this issue, the DNA fingerprints of strains isolated during the first and subsequent episodes have been compared.

The first use of this approach was on HIV-infected patients in a New York City hospital whose cultures were repeatedly positive for *M tuberculosis*. In some patients, the DNA fingerprint of the subsequent organisms remained unchanged despite the development of drug resistance. In other patients, the DNA fingerprint changed dramatically, demonstrating exogenous reinfection with a novel *M tuberculosis* strain.[15] Another study addressed the relative frequency with which reinfection and relapse occur in a population. By fingerprinting the bacteria from 698 people with tuberculosis diagnosed during 6 years in a community in South Africa, the authors identified 16 people (none of them coinfected with HIV) who had a new episode of tuberculosis following curative therapy. When the DNA fingerprints of the initial and subsequent strains were compared, 12 patients were found to have been exogenously reinfected.[16] These data show that patients are not completely protected from a subsequent episode of tuberculosis and that exogenous reinfection may account for much of the disease in some regions. This observation reinforces the need for strong control programs to interrupt transmission, even in settings where high percentages of people are already infected.

RESTRICTION FRAGMENT-LENGTH POLYMORPHISM ANALYSIS CAN HELP IDENTIFY FALSELY POSITIVE CULTURES

An unanticipated finding of the molecular epidemiologic studies has been that an appreciable number of cultures are falsely positive. In investigations of people with the same strains of *M tuberculosis*, evidence of disease was unconvincing; rather, they seemed to have cultures that were positive because of contamination in the laboratory.[17] This can occur through mislabeling and contamination of reagents and the transfer of *M tuberculosis* between specimens. Suspected cross-contamination begins either with the clinician (when there are inconsistencies between clinical and microbiologic results) or in the laboratory (when there is an inappropriately large number or pattern of positive cultures). Population-based studies suggest a prevalence as high as 3%.[18] The consequences of laboratory cross-contamination are serious. Patients and contacts are subjected to additional physician consultations and tests, unnecessary treatment, and delays in the diagnosis of other diseases. Because bacterial DNA fingerprinting is an indispensable tool for confirming suspected cross-contamination, the Centers for Disease Control and Prevention have made this test available by contacting local tuberculosis control personnel.

EFFICIENT TUBERCULOSIS CONTROL PROGRAMS DECREASE TRANSMISSION

The cohort evaluation of patients with tuberculosis and the trends in the incidence and mortality have been the main measurements to evaluate the control programs.[19] Currently, a molecular epidemiologic approach is also being explored. In San Francisco, the tuberculosis control program intensified its activities in 1991 by improving contact investigation, expanding the use of direct observed or short-course therapy, developing an HIV-related tuberculosis prevention program, screening for tuberculosis and the use of preventive therapy among high-risk groups, and improving hospital infection control measures. Conventional epidemiologic data showed a decrease in the incidence from 51.2 cases per 100,000 people in 1992 to 29.8 cases per 100,000 in 1997; molecular surveillance showed a decrease in the rate of disease attributed to recent transmission from 10.4 cases per 100,000 in 1992 to 3.8 cases per 100,000 in 1997 ($P < 0.001$).[20] This result suggests that control measures have been particularly successful in interrupting disease transmission. Many people still have reactivation of their latent infections, however, illustrating the need to expand efforts to diagnose and treat latent infection.

CONCLUSION

Molecular epidemiologic approaches have provided novel insights into the transmission and pathogenesis of tuberculosis. But no one is safe from tuberculosis until we are all safe, and thus, the goal of eliminating tuberculosis in the United States remains essential for the long-term control of the disease.

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Practice point

Looking for the man who fell off the roof

Dr Mumps, a primary care physician, has a patient who fell off a roof and was taken to the University of Excellence Hospital, the one that advertises every morning on the radio. In a capricious mood, Mumps decides to find out how his patient is doing. He is greeted by a friendly recording: "If you speak Spanish, press one. If you speak Swahili, press two. To speak to the laundry, press three; for the kitchen, press four; for billing, press five. If you have exhausted all 12 possibilities, stay on the line. Your call is very important to us, and we will answer you promptly."

Half an hour later, Mumps at last speaks to a human voice. It directs him to Dr Buggs, the attending physician, an infectious disease specialist. Buggs answers promptly, but says that she has been "off service" for a week and is back in the laboratory. She suggests calling Dr Chan, offers to transfer the call, but inadvertently disconnects the phone.

Undaunted, Mumps tries again. Not once, but many times. Each time, he must endure the same recording: "If you speak Turkish, press seven . . . " He looks for Dr Chan, but there are 25 Chans working at the University of Excellence. On the third attempt, he finds the right one, but he has signed off the day to Dr Patel, who says that the patient belongs to Dr Mbawa, who believes that the patient belongs to Dr Rigamortis, who is doing a venepuncture and cannot come to the phone. Mumps asks for the patient's room: there is no reply. Perhaps she has gone for an x-ray examination.

The next day, Dr Rigamortis does not answer. The nurse is "on his break." The medical student seems to be in charge, but does not know the patient and suggests calling medical education. Alas, the program director is in a meeting that will last all day. The secretary is "away from her desk" but calls back later. Dr Rigamortis has gone for a job interview. His patients are being seen by Dr Mbawa, she adds mournfully, but the good doctor fell off the roof last night and is in intensive care. "You could call the new attending physician, Dr Smith," she muses, "but he has gone off to a meeting in Patagonia, and Dr Buggs is covering for him."

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