The Timeless Legacy of Robert Koch

Founder of Medical Microbiology

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Robert Koch was a German physician and scientist who made wide-ranging contributions to the study of infectious diseases. He is credited with developing medical microbiology as a new and independent discipline. He is most renowned for deciphering the etiology of tuberculosis (TB), anthrax and cholera. He laid down rigorous guidelines for establishing the link between a pathogen and a particular disease that came to be known as Koch's postulates. To this day these postulates serve as benchmarks in the study of infectious diseases.

The year 2005 marked the 100th anniversary of the Nobel Prize awarded to Robert Koch for his discovery of the tubercle bacillus, the causative agent of TB, a disease that was rampant in Europe towards the end of the 19th century. The significance of Koch's seminal discovery of the TB bacillus is only overshadowed by his overall contribution to the study of infectious diseases. What were the events and circumstances that triggered Robert Koch's interest to study infectious diseases including TB?

Early Life

Koch was born in Clausthal, Germany in 1843, the son of a mining engineer. He astounded his parents by teaching himself to read at the age of five. After a successful stint at school where he showed an interest in biology, he attended the University of Göttingen, Germany, during 1862–1866 where he studied mathematics, natural sciences and later, medicine. During that period he was most fortunate to be exposed to the 'infectious disease' hypothesis of his teacher Jacob Henle (1809–1885), professor of anatomy and physiology. Henle had proposed that

Keywords
Koch's postulates, tuberculosis, anthrax, medical microbiology.

germs, the 'hidden contagion', were responsible for causing many diseases. A similar role for germs in fermentation and putrefaction had been proposed by Louis Pasteur (1822–1895) of France in 1860. These were significant contributions as it was believed at that time that fumes ('miasma') generated during the process of degradation and putrefaction actually caused disease. A counterview to the germ theory of disease was proposed by Rudolf Virchow (1821–1902), a reputed pathologist from Berlin. Virchow's theory was that all diseases originated within the body's cells themselves. Henle's teachings at the medical school probably left an indelible impression on Koch's mind and were to guide his future search for 'hidden contagion'.

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Success with Anthrax

When Koch commenced his medical career as a District Medical Officer in Wollstein at approximately 30 years of age, he was determined to explore the relationship between disease and microorganisms (now called pathogens). He set aside some space in his own home for a small laboratory to perform his experiments on anthrax which included a microscope gifted to him by his wife, Emmy Fraats. He had no formal research training and since he was posted in rural Germany he was physically removed from the scientific and research centres of the times. While this isolation perhaps allowed Koch to have independence in scientific thought, he was handicapped by a lack of peer review and certification. He was astutely practical to realize that his experimental findings would have to be authenticated by a peer scientist to be accepted by the scientific and medical community; so he associated himself with Ferdinand Cohn (1828–1898) at the Institute for Plant Physiology, University of Breslau (present day Wroclaw in Poland). Koch conceived and executed a new approach to study the relationship between disease and microbes. Thorough examination of the tissues from experimental animals, bearing in mind the ecological context of the epidemic in humans, visual representation of the microbes became part of this original and successful strategy.

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The first disease that Koch studied was anthrax, which caused mortality in cattle and sometimes in humans as well. What made Koch chose anthrax as a subject to study the relationship between germ and disease? Was it a serendipitous choice? Or was his interest spurred by the fact that many intriguing features of anthrax were already known at that time including Pollender's discovery of rod-shaped bodies in the blood of cows dying of anthrax? Davaine had demonstrated that the disease could be transmitted to healthy animals by inoculating them with blood that contained these bodies and Cohn showed that the bacteria can form spores that re-form into bacteria after a resting period. Even so, one cannot but marvel at Koch's experimental design to study anthrax. His use of mice as an experimental model allowed him to investigate the disease under quite reproducible conditions in a laboratory set-up. A high bacterial count in the blood (bacteremia) was a feature in infected animals. The happy circumstance (for the experimentalist!) that the bacilli were relatively large made their microscopic demonstration in the blood easier. Along with Cohn, he elucidated the life cycle of *Bacillus anthracis*. They used a simple microscope to examine the organisms cultured *in vitro* and from tissues and added their drawings to the original publication on anthrax as scientific proof. Koch succeeded in demonstrating the infectivity of spores derived from pure cultures of the anthrax bacillus and that animals infected with these spores died of anthrax. Thus he established the etiology of anthrax and proved that the anthrax bacillus could form spores and in turn spores could form bacilli. This became a fundamental concept of bacteriology and highlighted the importance of spore forming bacteria from soil in medical microbiology. With the publishing of this meticulously carried out work in 1876, Koch came into the limelight in Germany. In 1880 he moved to the Imperial Health Office in Berlin where he was provided with a decent laboratory and two research assistants.

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Koch developed several techniques that were instrumental in establishing bacteriology as an experimental science. He introduced the solid medium for bacterial cultivation, first using gelatin and subsequently agar to solidify the medium on a flat dish invented by his colleague Petri, which is still the pet tool of microbiologists. The methodology of disinfection and sterilization was standardized by him. He also perfected the methods of bacterial staining so that bacteria could be visualized more easily and photographed. The overall result was a substantial improvement in the ability to obtain pathogenic bacteria in pure form and to detect, identify and document them easily.

Focus on TB

At Berlin, he began to focus on TB, which was a major health threat in Europe towards the end of the 19th century. In 1865, the French army doctor Jean Antoine Villemin showed that TB could be passed from humans to cattle and from cattle to rabbits. He postulated that a specific microorganism was the causative agent of TB. The stage was set for detective Koch. He applied the tenets that had worked in the case of the anthrax bacillus to the study of TB. However the techniques that were successful with anthrax failed in the study of TB. So he innovated on the staining technique to visualize the TB bacillus in experimentally infected animals and correctly concluded that the bacillus possessed "a special wall of unusual properties". He keenly observed that bacteria were characteristically arranged in bundles and that sections containing TB and leprosy bacilli were similar. He designated them tubercle bacillus. We now know that the TB and the leprosy bacilli are both mycobacteria and both are intracellular pathogens. Koch was a rigorous experimenter and rational thinker and did not assume that the mere presence of the TB bacillus indicated that it was the cause of the disease. Therefore he embarked on isolating the organism in pure form from infected tissues. Here he met with frustration initially as the culture techniques which worked in the case of anthrax did not work well for TB. He finally succeeded in culturing the organism using solidified blood serum after patiently waiting for more than ten days. Now we know that the TB bacillus is a slow grower and could have escaped detection had Koch not

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persisted in incubating the inoculated culture medium for longer. The missing link between pathogen and disease was established when he demonstrated that TB with similar pathology developed in guinea pigs infected with the *in vitro* cultured bacteria as that observed in animals inoculated with human-derived material. The results of these meticulously conducted experiments were presented by Koch at the meeting of the Physiological Society of Berlin on March 24, 1882. He received wide scientific acclaim for this work and March 24 is observed as World TB day each year to commemorate the momentous announcement of his discovery. The strict criteria devised by Koch to establish the link between a microorganism and disease are known as Koch's postulates and are even today applied in the case of infectious diseases.

Koch's Studies on Cholera

In 1883, another infectious disease beckoned Koch when he led a German expedition to Egypt and India. He succeeded in isolating *Vibrio cholerae* in Alexandria but moved on to Kolkata (then Calcutta) since the disease had subsided in Egypt and was endemic in India. It was here that he demonstrated the link between contaminated drinking water and cholera outbreaks. Koch and his colleagues received a heroes' welcome when they returned to Berlin in May 1884. It must be placed on record though that this honour was not entirely deserved because thirty years ago in 1854, an Italian scientist by the name of Filippo Pacini (1812–83) had described the comma-shaped bacteria which he called as *Vibrio*. Pacini's work was largely ignored by the scientific community. Robert Koch too was unaware of Pacini's work at the University of Florence and he independently discovered the cholera organism. It was not until 82 years after Pacini's death that in 1965 the organism was formally renamed as *Vibrio cholerae Pacini 1854*. Although Koch cannot be credited with discovering the cholera bacterium first, for his understanding that cholera spread through contaminated water, Koch was awarded a prize of 100,000 German Marks. His studies

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led to the introduction of hygienic measures including providing clean drinking water and public health measures that helped to control cholera outbreaks in Hamburg, Germany. In 1885, Koch was appointed Professor of Hygiene at the University of Berlin and also Director of the newly established Institute of Hygiene in the University there.

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Back to TB

Robert Koch now shifted the focus of his research to devising methods for the prevention and treatment of TB in what was to be the most controversial phase of his scientific career. In August 1890, he publicly announced at the 10th International Congress of Medicine at Berlin that TB in guinea pigs could be terminated by treatment with tuberculin derived from the TB bacillus. This announcement was received with great excitement by both the government and the public. A clinical trial was undertaken immediately with tuberculin on 1769 patients and in February 1891 the verdict was out. Tuberculin failed to have a protective effect; only 1% was cured while 34% showed some improvement, 55% showed no improvement and 4% had died. It is of interest that Koch had initially tested this material on himself, which would have been unacceptable in the present day, and recorded a strong positive reaction to tuberculin which is referred to as a delayed hypersensitivity test (DTH). His hasty announcement of a treatment for TB without thorough laboratory evaluation did not match his scientific rigour to establish the link between pathogen and disease. Was this likely due to the immense societal and /or political pressures he faced to produce a cure for TB which was a life threatening disease? Perhaps history would not judge Koch too harshly for his failure to cure TB which remains a daunting challenge to this day. We still do not have a preventive treatment for TB that is derived from bacterial products. However Koch predicted that tuberculin would be a useful diagnostic tool and indeed the tuberculin or Mantoux test (called after Charles Mantoux who followed up on Koch's findings) is used to this day to detect exposure to TB in non-endemic disease settings.

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Koch, a Successful Mentor

Despite his failure to cure TB, he retained his standing among his scientific peers. In 1891 he became an Honorary Professor of the Medical Faculty of Berlin and Director of a new Institute for Infectious Diseases that was opened for him in Berlin which was later named as the Robert Koch Institute. Unraveling the mechanisms underlying infectious disease continued to be his lifelong passion. He nurtured several researchers some of whom became outstanding scientists in their own right, such as Friedrich Loeffler (1852–1915), discoverer of the causative agent of diphtheria and of the first filterable 'invisible' pathogen (foot and mouth disease virus) and Georg Gaffky (1850–1918), co-discoverer of the cholera organism with Koch. Other members of the Koch Institute focused on the host immune response to disease which led to the birth of immunology as a new discipline of medical microbiology. Noteworthy amongst these were Emil von Behring (1854–1917) who discovered passive immunization against diphtheria and tetanus and was awarded the Nobel Prize ahead of Koch in 1901; Shibasaburo Kitasato (1852–1931) co-discoverer of passive vaccination against diphtheria with von Behring and discoverer of the plague bacterium, *Yersinia pestis*; Paul Ehrlich (1854–1915) who proposed the side-chain theory in immunology and discovered complement and developed the principles of chemotherapy (Nobel Prize in 1908) and August von Wassermann (1866–1925) who developed the immunodiagnosis of syphilis. The productivity and originality of the institute's research was recognized by the award of three Nobel Prizes to its scientists.

Koch's Work on other Diseases

In 1896 Koch traveled to South Africa to study rinderpest and although he did not identify its cause (now it is known to be caused by a virus which could not have been cultivable using the classical bacteriological techniques), he used bile therapy to contain an outbreak of the disease. Thereafter he studied malaria, surra of cattle and horses and plague, and published his findings on these diseases in 1898. Upon returning to Germany he embarked on a trip to Italy and the tropics. He successfully confirmed Sir Ronald Ross's findings on malaria (see *Resonance* July 2006) and contributed to the understanding of the etiology of the different forms of malaria and their control with quinine. In 1904, Koch traveled to German East Africa to study East Coast fever of cattle. He made important observations not only on this disease, but also on pathogenic species of *Babesia* and *Trypanosoma* and on tick-borne spirochetosis, continuing his work on these organisms when he returned home. He returned to Central Africa in 1906 to work on the control of human trypanosomiasis and reported on the effectiveness of atoxyl against this disease.

Recognition

Koch was the recipient of numerous medals and awards, honorary doctorates of the Universities of Heidelberg and Bologna,

Koch's Postulates

These were proposed in 1882 as the necessary conditions to prove that an organism is the cause of a particular disease:

1. The presence of the organisms must be demonstrated in every case of the disease.
2. The organism must be isolated from the host carrying the disease and grown in pure culture.
3. The specific disease must be reproduced when a pure culture of the organism is inoculated into a healthy susceptible host.
4. The organism must be recoverable from the experimentally infected host.

Koch's postulates have limitations and may not always be valid. For example, in some cases, the microorganism (such as *Mycobacterium leprae* that causes leprosy) cannot be grown in pure culture in the laboratory. There is also no animal model of infection with *M. leprae*. At times, "harmless" organisms such as *E. coli* may cause disease if they acquire extra virulence factors that make them pathogenic. Similarly organisms that are normally non-virulent may cause an infection in immuno-compromised individuals such as those suffering from AIDS. Also not all hosts infected by a bacterium may develop disease. Sub-clinical infection is usually more common than clinically obvious infection.

Notwithstanding these limitations, Koch's postulates still offer a useful yardstick in establishing a causal relationship between a microorganism and a disease.

honorary citizenships of Berlin, Wollstein and his native Clausthal, and honorary memberships of learned societies and academies in Berlin, Vienna, Posen, Perugia, Naples and New York. He was awarded the German Order of the Crown, the Grand Cross of the German Order of the Red Eagle (the first time this high distinction was awarded to a doctor), and Orders from Russia and Turkey. He received many awards posthumously in several countries. In 1905 he was awarded the Nobel Prize for Physiology or Medicine for his studies relating to TB.

Robert Koch was married twice. In 1866 he married Emmy Fraats and fathered one daughter, Gertrud. In 1893 Koch married Hedwig Freiberg after divorcing his first wife. Koch died of heart failure on May 27, 1910 in Baden-Baden, Germany. But his postulates are timeless since they were based on rigorous scientific criteria of hard evidence, association and reproducibility. Over the years, his postulates have been modified to accommodate organisms and diseases unknown at the time of their formulation in 1882, such as viruses and obligate parasites.

Concluding Remarks

Robert Koch's passion was studying infectious diseases and he identified the etiological agent for anthrax, TB and cholera. He established medical microbiology as an independent scientific discipline whose cornerstones were experimentation and visual documentation. He formulated postulates which had to be experimentally verified before a pathogen could be unequivocally linked as the cause of a particular disease. The Koch's postulates are now being used in their 'molecular' avatar to establish the connection between gene function and pathogenicity (Falkow, 1988). The establishment of medical microbiology as a solid and independent discipline led in turn to the study of the associated host response and the emergence of immunology.

Suggested Reading

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