

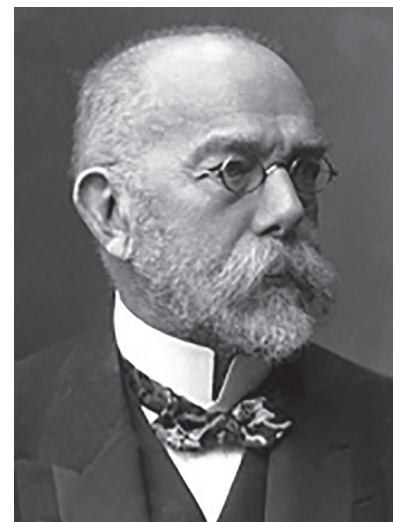
REVIEW ARTICLE

Founder of Modern Bacteriology: Robert Koch

INTRODUCTION

The celebrated German doctor Robert Koch was one of the most important and influential bacteriologists in history. He is considered the founder of “modern bacteriology.” Koch is credited with developing many innovative and fundamental techniques – some of which are used today – and proving that microorganisms caused anthrax, cholera, and tuberculosis. His work was essential in proving the germ theory of disease and that such diseases were contagious. He was instrumental in applying the germ theory to community health and hygiene practices in order to prevent diseases in his native Germany and elsewhere. Koch won the Nobel Prize for Physiology/Medicine in 1905 and received many other medals and honors during his lifetime and after his death.

Robert Heinrich Herman Koch, a celebrated German physician and pioneering microbiologist, is also known as the founder of modern bacteriology. He is also known for his role in identifying the specific causative organisms of tuberculosis, anthrax, cholera and also for rendering experimental support for the concept of infectious diseases. In addition to his innovative studies in these diseases, Koch created and improved laboratory technologies and techniques in the field of microbiology and also made key discoveries in the field of public health. His research led to the creation of Koch’s postulates, a series of four generalized principles linking specific microorganisms to specific diseases that remain the “gold standard” in medical microbiology today.^{1,2}



EARLY LIFE AND MEDICINE

Robert Koch was born in Clausthal, a silver mining town, in Hanover, North West Germany, on December 11, 1843, to Herman Koch and Mathilde Julie Henriette Biewand. He was the son of a mining engineer and the third of 13 siblings. He was a precocious reader with an aptitude for science and maths. Right from an early age, he was excellent in academics. At the age of 19, Koch entered the University of Gottingen to undergo medical studies and was under the influence of the world famous anatomist Jacob Henle who had published the theory of contagion in 1840. In his sixth semester, he carried out research studies at Physiology Institute, where he studied succinic acid secretion, and this eventually led to the basis of his dissertation. In 1866, Koch graduated from medical school, earning honors of highest distinction. He learned under the tutelage of Jacob Henle, Rudolf Vircho, George Meissner, Friedrich Wohler, and Welhelm Krause. His famous disciples were Paul Ehrlich, August von Wasserman, and Emil von Behring (who won Nobel Prize for diphtheria antitoxin). While a student, he won a research prize for his study on neuronal intervention of the uterus, which was carried out under the guidance of Henle. This allowed him to travel to Hanover, when he encountered Germany’s most renowned physician, Rudolf Virchow.¹

In the year 1866, he qualified maxima cum laude with a master’s thesis on succinic acid. After his graduation, he was in general practice at Rakwitz for some time. In 1867, Koch married Emmy Fraatz, a childhood friend, and they had a daughter Gertrude, but his marriage broke after 26 years. In 1893, he married a young actress Hedwig Freiburg.^{1,2}

In 1871, he served the German Army as a surgeon in the Franco-Prussian war and later on worked as a physician in Wolstein, Poland. In 1872, after discharge from the army, Koch became district medical officer for a rural area near Berlin. His wife gifted him a microscope on his birthday and by making a small (primitive type) laboratory at the back of his house, he started experiments with microbes.²

In 1879, Koch was appointed as district physician at Breslau, Poland. The next year (1880), at the invitation of Imperial government, he joined the staff of Imperial Office and developed plate culture technique. Between 1885 and 1890, he served as administrator and professor of hygiene/bacteriology at Berlin University and director of Hygiene Institute. There, Koch attracted a host of exceptionally gifted coworkers: Loeffler, Gaffky, Eberth, von Behring, Pfeiffer, Welch, Kitasato, Ehrlich, Wassermann, and others.^{1,3}

KOCH'S CONTRIBUTION TO THE FIELD OF TUBERCULOSIS

Discovery of Tubercle Bacillus (1882)

In 1880, during his work as the government advisor with the Imperial Department of Health in Berlin, Robert Koch became interested in tuberculosis research. In those days, people used to believe that tuberculosis was an inherited disease. However, Koch was convinced that the disease was caused by a bacterium and was infectious, and tested his four postulates using guinea pigs. He is also known as the father of scientific approach to the management of tuberculosis or father of scientific study of tuberculosis.^{1,3-5}

Koch's lecture is considered by many to be the most important in medical history; it was so innovative, inspirational, and thorough that it set the stage for the scientific procedures for the 20th century. On March 24, 1882, Koch presented his findings on tuberculosis in a monthly meeting of the Berlin Physiological Society. His demonstration included more than 2,000 microscopic preparations, now regarded as one of the most influential presentations in medical history. He described how he had invented the new staining method and demonstrated for the audience. Koch brought his entire laboratory to the lecture room: Microscope, test tubes with cultures, glass slides with stained bacteria, dyes, reagents, glass jars with tissue samples, etc. He offered the audience to check his findings themselves. He showed tissue dissections from guinea pigs that were infected with tuberculous material from the lungs of infected apes, from brain and lungs of blood-borne tuberculosis, from the cheesy masses in lungs of chronically infected patients, and from the abdominal cavities of cattle infected with tuberculosis. In all these cases, the diseases that had developed in the experimentally infected guinea pigs were the same, and the culture of the bacteria taken from the infected guinea pigs was identical. The renowned scientist and Nobel Laureate later confessed, "I hold that evening to be the most important experience of my scientific life." When Koch finished his talk, there was a complete silence. No questions, no congratulations, no applause. The audience was stunned. By and by, the people got up and started looking into the microscope to visualize the tuberculosis bacteria with their eyes.³⁻⁶

WORLD REACTION AFTER THE DISCOVERY OF TUBERCLE BACILLUS

On April 10, 1882, Koch published the lecture in *Berliner Medicinische Wochenschrift*, under the title "Die Aetiologie der tuberculosis." The mysterious disease which had killed millions of humans as well as animals especially cattle was no more a secret. Apart from the journals, his discovery hit the headlines of world media and press. Prof John Tyndal published the essential findings in the "*Times*" on April 2, 1882. *New York World*, *New York Times*, *New York Tribune*, and other world newspapers covered the discovery. Koch had overnight become almost a household name and a world figure, and "Koch bacillus and Koch's disease entered the medical jargon."^{1,2}

Staining of Tubercle Bacillus

Koch preferred methylene blue as stain and caustic potash was added to it. When the cover glass was exposed to the staining for 24 hours, very fine rod-like forms became apparent in the tubercular mass for the first time, and the power of multiplication and spore formation was also observed.

- Paul Ehrlich used the mixture of gentian violet and aniline oil. He observed acid fastness of bacilli.
- Franz Ziehl: Recommended carbolic acid, instead of aniline oil.
- Friedrich Neelsen: Recommended Fuchsin and sulfuric acid, instead of gentian violet and nitric acid. In this way "Ziehl Neelsen stain" and "acid fast bacillus" were born.^{1,2,3,7}

Culture of Tubercle Bacillus

Koch's postulates required coincidence of bacteria and disease, isolation of bacteria in pure, and induction of disease by inoculation with bacteria from pure culture. After experimenting with different media at different temperatures, he observed that the colonies grew on coagulated blood serum at 37 to 38°C. The colonies were dry and scaly and appeared only in the second week of incubation. No other bacterial species had this appearance or grew so slowly. To test the pathogenicity of bacteria, he inoculated 217 animals with bacilli from pure culture. In every case, tubercles appeared in number proportional to the size of inoculums. Tubercles did not appear after the injection of nontuberculous tissue. Koch concluded that tubercle bacillus was the cause of disease and its defining characteristics.^{1,3,6-8}

Koch detected the bacilli in the sputum and lung cavities of the consumptives. By inoculating healthy animals with infected sputum, he could induce disease and thus concluded that sputum was the principal source of

transmitted disease, and patients with pulmonary and laryngeal tuberculosis, expectorating bacilli, were particularly infectious.^{1,9,10} Although bacilli would not multiply outside a living host, in dried sputum they retained their pathogenicity for weeks. Proper disposal of sputum and decontamination of environment were, therefore, essential for disease prevention.

Koch's development of solid culture media was a major turning point. He observed that by placing liquid cultures in gelatin and then cooling the solution, a clear, smooth, homogeneous culture medium was able to be produced in which bacteria multiplied to form visible colonies.^{1,5,7}

Koch introduced "plate technique." He observed the growth of bacterial colonies and subjected them to steam and various chemicals, thus advancing the field of disinfection and sterilization. He noticed that while certain chemicals killed bacteria, others merely inhibited them – a distinction that would be important in the antibiotic era. In a manuscript published in 1881, he described his plate technique in exquisite detail. Others could now replicate. Koch's paper became the "Bible of bacteriology."^{3,6,7}

Koch and Tuberculin

In the 10th International Medical Congress held in Berlin in 1890, Koch read a paper on "Bacteriological Investigation." He dropped a bombshell by announcing that he made a substance that hindered the growth of mycobacteria, cured tuberculosis in infected guinea pigs, and would probably be useful in human tuberculosis. This discovery sparked interest throughout the world and was published in "*Lancet*" and "*British Medical Journal*." The substance came to be referred to as "Koch's lymph," but Koch named it as "Old tuberculin." Thousands of people including the English physician and writer Sir Arthur Conan Doyle and Sir Joseph Lister visited him. In the United States, dollars was offered for a teaspoon of remedy. But it did not provide any cure; rather, it worsened the disease in some patients. High hopes were followed by sad disappointments. Koch defended himself by maintaining that he had never claimed a universal cure for all cases of tuberculosis. In 1907, Koch produced a better version known as new tuberculin, first tuberculin residue, and later bacillary emulsion. However, tuberculin was to prove to be of enormous value as a diagnostic tool to distinguish the infected from noninfected, especially when Bacillus Calmette–Guérin vaccination was later introduced. Moreover, the study of tuberculin reaction paved the way for subsequent research on allergy and immunology.^{1,5,6,10}

Human vs Bovine Tubercle Bacillus Controversy

Koch first described the tubercle bacillus in 1882 and considered that human and bovine bacilli were separate organisms, as established by Villenin in 1865. In the same year, he concluded that human and bovine bacilli were identical; but despite the fact that bovine tuberculosis was transmissible to humans, he did not feel that bovine infection was an important human hazard.

On this controversy, the Privy Council set up a Royal Commission on Tuberculosis and its final report (1911) disapproved Koch's alleged views, which has obviously underestimated the seriousness of bovine tuberculosis as public health hazard. The commission had recommended the preventive measures including the eradication of infected cattle and pasteurization of milk.¹

Koch's Postulates

Koch's postulates are based on four criteria, designed to establish a causative relationship between a microbe and disease. The postulates were formulated by Robert Koch and Friedrich Loeffler in 1884, based on earlier concepts described by Jacob Henle, refined and published by Koch in 1890. Koch applied the postulates to describe the etiology of cholera and tuberculosis, but they have been controversially generalized to other diseases.

Postulates

- The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.
- Microorganisms must be isolated from a diseased organism and grown to pure culture.
- The cultured microorganisms should cause disease when introduced into a healthy organism.
- Microorganisms must be isolated from the inoculated diseased experimental host and identified as being identical to the original specific causative agent.^{1,6-8}

Koch's Phenomenon

Koch's phenomenon is the response of tuberculous animal to reinfection with tubercle bacillus marked by necrotic lesion that develops rapidly and heals quickly and caused by hypersensitivity to products of tubercle bacillus.

- The already infected guinea pigs are injected with live tubercle bacilli.
- The injected site becomes necrotic.
- The same reaction was observed by Koch, when the animal was injected with old tuberculin.
- It had produced the same reaction.
- This is termed as Koch's phenomenon.
- The test represents the clinical application of type IV (delayed type) hypersensitivity reaction.

Koch's phenomenon or the necrotic reaction is known to be due to overproduction of proinflammatory cytokines (particularly tumor necrosis factor α). The fact remains that inflammatory response is required for successful protection against tuberculosis.^{1,5,6}

Discoveries of main bacterial pathogen by Robert Koch¹:

| Year | Organisms | Disease |
|------|-----------------------------------|--------------|
| 1877 | <i>Bacillus anthrax</i> | Anthrax |
| 1878 | <i>Staphylococcus</i> | Suppuration |
| 1882 | <i>Mycobacterium tuberculosis</i> | Tuberculosis |
| 1883 | <i>Vibrio cholerae</i> | Cholera |

Koch's Contribution in Other Fields: Milestones of Achievements in his Life

- Anthrax (1876): Koch was the first to link a specific bacterium with a specific disease, and the first disease that Koch investigated was anthrax. In a makeshift laboratory at the back of his house, he had elucidated the life-cycle of anthrax bacillus. Anthrax was seriously affecting herds of farm animals and the farmers were afraid of it. In 4 years of working as district medical officer, he initiated studies in a major health problem, anthrax, that had killed 528 people and 56,000 livestock. He did inoculation studies using mice, rabbits, guinea pigs, dogs, frogs, and birds. He observed that inoculation of a mouse with infected sheep blood, which died of anthrax, caused the mouse to die of anthrax. At autopsy, rod-shaped structures were present in the blood, lymph node, and spleen. By repeating these inoculations, Koch could propagate anthrax rods over dozens of generations. He hypothesized that these were living bacteria that propagated by elongation and fission. He studied its lifecycle from the spores, proved the etiopathogenesis, cultured the germs, and determined the conditions under which its spores develop and spread and put forward effective proposals for preventing the transmission of disease. Koch's discovery of anthrax bacillus launched the field of medical bacteriology.^{1,5,6,10,11}
- In 1878, while studying wound infection in animals, with the technique of identifying bacteria, he observed various disease states at microscopic level, such as septicemia, abscess, and gangrene. He submitted his views that bacteria exist in different species producing clinical syndromes and he discarded the notion that bacteria with different morphologies were derived from the same species.⁶
- Developed plate technique for generating pure culture of bacteria. This technique is regarded as one of the greatest contributions to bacteriology. The development of solid culture media was a major turning point. On a slice of potato, the growth of fungal colonies was observed, and each colony was pure, containing similar organisms. The culture medium was clear, smooth, homogeneous, and bacteria multiplied to form visible colonies. He also subjected them to steam and chemicals.^{1,6}
- Cholera was nicknamed as "King Cholera" or "White Death" and was the most dangerous killer disease in the late 19th century. In the year 1883, Koch headed German Cholera Commission in India and Egypt, where he isolated *Vibrio cholerae* as the cause of cholera in Egypt. By histopathological studies, he successfully demonstrated that the pathological lesion was confined to intestinal mucous membrane. He also discovered that the bacilli were responsible for Egyptian ophthalmia.^{1,5,6,10,11}
- In 1881, he laid the foundation of modern bacteriological techniques by:
 - Introducing glass slide and cover slip
 - Examination by hanging drop
 - Fixing and staining of bacteria

- Culture on solid media by poured plate method
- Microphotography of the microorganisms

He demonstrated his bacteriological techniques and created a sensation. Even Pasteur exclaimed: "C'est un grand progress, Monsieur i."^{3,6,10}

- During these techniques, he demonstrated *Streptococci* and *Staphylococci*, the causative organisms of wound infection.^{1,6}
- He invented disinfection by steam sterilization.⁶
- In East Africa, he uncovered the cause of a mysterious fever in Usumbra Mountain, as due to malaria.⁶
- He showed that trypanosomes, the causative organism of sleeping sickness, undergo a cyclical phase in tsetse flies.⁶
- He carried out basic studies into disease resulting from wounds.
- For the first time, he showed that certain disinfectants are bactericidal rather than bacteriostatic and also maintained that the different organisms differ in their susceptibility to various disinfectants.^{1,6}
- In 1978, he identified the germ that caused blood poisoning and septicemia. He discovered that methyl violet dye revealed the septicemia germ under a microscope by staining it. He also photographed the germs so that the people outside the laboratory could see them.^{1,5,6}

VISITS AND HONORS IN OTHER COUNTRIES

In the year 1875, Koch visited many of Germany's great scientific centers, which attuned him to emerging world of microbial science. In 1883, Robert Koch headed German Cholera Commission in Egypt and India, where he isolated cholera vibrio, as the cause of cholera.¹² In 1885, Koch was elected Professor of Hygiene and Bacteriology in Berlin, and Koch Institute was built for him. Koch traveled a lot to study the tropical diseases of Africa, Asia, and other countries. In 1897, he was elected foreign member of Royal Society (ForMemRS). The same year, he came to India and visited many important institutions including Mukteswar in Uttarakhand Hills, a campus of Indian Veterinary Research Institute, Bareilly. Upon his return from India, he was honored by Kaiser Wilhelm 1 and Chancellor Otto von Bismark. Koch also visited Italy, Indonesia, New Guinea, the USA, and Japan. In Japan, he was greatly honored by his old student and associate Kitasato at his own Institute of Bacteriology and a Shinto shrine was erected there in his honor, and even now, the annual tribute is being paid to him. Even after his retirement from the Institute of Infectious Diseases, Koch continued to be universally honored and was elected member of German Academy of Sciences and was later elevated to the title of Excellency. Koch's name is one of 23 from the field of hygiene and tropical medicine building in Keppel Street, Bloomsbury. A large statue of Koch stands in a small park known as Robert Koch Platz just north of the charity hospital, in the Mitte section of Berlin.^{1,6,9,10}

Koch suffered from a heart attack on April 9, 1910 and never made complete recovery. On May 27, 1910, at the age of 67, only 3 days after delivering a lecture on his tuberculosis research at the Prussian Academy of Science, Koch died at Baden. The institute was named after him in his honor.^{1,6}

CONCLUSION

It can be very well concluded that any single discovery of Robert Koch – the discovery of tubercle bacillus being outstanding – would have ensured a place in medical history. But when one considers his total outstanding achievements, he stands out as one of the medical giants of all times. Medicine and humanity at large remain forever indebted to him.

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