

Pasteur and Koch: the fathers of microbiology

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ABSTRACT

Introduction: scientists Louis Pasteur and Robert Koch made transcendental contributions to medicine, to science in general, and in particular to microbiology, many of which transmit an irrefutable and elevated knowledge. **Objective:** to describe the work carried out by Pasteur and Koch in the field of microbiology. **Method:** a bibliographic review was carried out in the period from November to December 2020 using as information resources those available through the Infomed network and the PubMed, SciELO and LILACS databases. The terms used in the search were: "Bacteriology", "History of medicine" and "Tuberculosis" and the following search strategy was formed: [(Bacteriology) AND (History of medicine) AND (Tuberculosis)]. **Development:** the contributions of these great scientists marked a before and after in the field of microbiology; with their discoveries, they established postulates and theories that would later constitute the bases for the study of pathogenic agents that cause diseases. From these studies, it was possible to develop preventive therapies since most of the causal agents were known. **Conclusions:** the formulation of the germ theory or microbial disease theory was the greatest insight in the investigations of both.

Keywords: Bacteriology; History of Medicine; Tuberculosis.

For man, research and discoveries have been fundamental, which have enabled the understanding of macro and microscopic life and the evolution and development of scientific thought.

Microbes represent the primary link in the evolutionary chain of the biological world and, although some were first seen by Anton Van Leeuwenhoek in the 17th century, the emergence of the simplest forms dates back three and a half billion years in the Archean era¹.

Microbiology is a specialized science, which deals with the study and analysis of microorganisms. Its historical development is divided into three periods: the discovery of the microbial world, with a predominance of the speculative era, the observational period, or spontaneous generation, where it is stated that living beings are formed from inert matter and the third period or the fermentation period marks the beginning of the cultivation of microorganisms where Pasteur, Lester

and Koch studied the role and behavior of bacteria in disease².

The 19th century, with the French Revolution in the political order, was also a century of scientific fruitfulness and modern medicine was one of its great creations².

In this context Louis Pasteur and Robert Koch arise, creators of the theory of germs as the cause of infectious diseases and the true founders of microbiology and immunology. Knowing the causal agent and how the disease developed, changed the mentality of investigating the diagnosis-cause towards the search for the destruction of the etiological agent^{3,4,5}.

It is considered that the germ theory is also the starting point for the so-called "molecular disease" or "molecular epidemiology" that allows diagnosing an infectious disease from its primary biological elements, examining its molecular components and its population genetics.

The beginnings of bacteriology were characterized by the rivalry between the French and Germans, headed by Pasteur and Koch, respectively, which was fruitful for medicine^{6,3}. Motivated by the relevant contributions of these men to science, this review is carried out with the aim of describing the work carried out by Pasteur and Koch in the field of microbiology.

METHOD

An exhaustive bibliographic review was carried out in the period from November to December 2020, using as information resources those available through the Infomed network and the PubMed, SciELO and

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Conflict of interests

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LILACS databases. The terms used in the search were: "Bacteriology", "History of medicine" and "Tuberculosis" and the following search strategy was formed: [(Bacteriology) AND (History of medicine) AND (Tuberculosis)]. Literature published from 2010 onwards was selected, for which a total of 23 bibliographic references were reviewed after checking the quality, reliability and methodological validity of the selected articles.

DEVELOPMENT

In the history of humanity there have been people who have influenced their peers, for their contributions to knowledge, for their determination to dominate the environment and put it at the service of human beings. Examples of this are Robert Koch and Louis Pasteur who in their practices even exposed themselves to the risk of contagion.

Robert Koch

The immense development of the productive forces unleashed by capitalism also has its expression in the area of health. Virchow revealed the cell as the sole constituent of living organisms, plants and animals, commerce discovered the microscope as a result of its needs, and dyes made their appearance in the biological field. A German scientist, Robert Koch, tenaciously studied tuberculosis based on the avalanche of knowledge produced at the time. Heinrich Hermann Robert Koch, was born on December 11, 1843. He was born in Clausthal-Zellerfeld, Baden-Baden, on the slopes of the Black Forest in Germany^{7,8,9}.

Germany has always considered being the birthplace of this distinguished scientist, a fact of prestige for the nation. He studied Medicine at the University of Göttingen and after graduation he practiced in Hamburg and Lagenhogen. Between 1872 and 1880 he worked as a country doctor in East Germany, where he began his scientific career as a bacteriologist^{10,11}.

His origins were very humble and surely not even Koch himself could have imagined at that time the magnitude of the work that he was about to accomplish.

His first contribution to microbiology was the isolation of *Bacillus anthracis*, the etiological agent of anthrax, in 1876-1877. He isolated the germ, cultivated it, and by inoculating it into animals reproduced the disease. It was the beginning of the bacteriological era. The first that applied the scientific method for the verification of a pathological phenomenon was Koch, who put into practice the principles of the method for the demonstration of the relationship between microorganisms with specific diseases. The experiment sought to demonstrate the pathogenic potential of a microorganism discovered by him

in blood cells of cattle affected by anthrax, today called *Bacillus anthracis*^{11,12,13}.

In Koch, a stoic and persevering character develops early on, which is consolidated by long hours of work in the laboratory and resigning of mundane pleasures; he is dedicated to understanding the world of microorganisms to fight against diseases and favor his contemporaries.

This scientist inoculated a mouse with the blood of an animal sick with anthrax; which sickened and died; suspected microorganisms were present in the rodent's blood. Later, he took twenty healthy mice and inoculated them with this blood. Each of the twenty mice died under similar circumstances as the first. He then took blood from each of the mice that died and cultured it on artificial media. The cultures grew a microorganism morphologically similar to that observed in the blood of the rats. These bacteria were inoculated into healthy mice and the same disease was produced^{11,12,13}.

This bacteriologist is considered the forerunner of the biomedical research model, this is a rigorous, intentional, systematic, objective, reproducible and predictive process.

In 1880 he was appointed a member of the Imperial Committee for Health in Berlin and in 1882 he succeeded in isolating the etiological agent of tuberculosis: *Mycobacterium tuberculosis*. Tuberculosis is one of the most terrible diseases that affect human beings, its antiquity is estimated between fifteen thousand to twenty thousand years. The merit was great, because *Mycobacterium tuberculosis* is a bacterium that requires special staining techniques and culture media other than those commonly used in bacteriology^{11,14,15}.

Mycobacteria are not easy microorganisms to work with in the laboratory, they stain with specific dyes and regarding growth in culture media, it is difficult to achieve, they require media with multiple nutrients and special compounds, so it was worthwhile overcoming all these obstacles at that time.

Koch proved to be a genius in bacterial staining methods, which finally enabled him to isolate the tuberculosis bacillus, he managed to establish the first techniques in culture staining of the microorganism with the use of aniline and methylene blue to stain dry smears, which he fixed on a slide for later documentation, for which he also started the microbiological record. He demonstrated by means of staining that in the sputum of tuberculosis patients there was an organism with special characteristics. He postulated norms that determine whether a microorganism is causally related to a disease, by enunciating a series of procedures and rules to isolate the tuberculosis bacillus and the cholera vibrio^{2,4,16}.

It is considered that all these ideas served as pillars to understand the pathogenesis and clinic of infectious diseases, and paved the way for the application of etiological treatments.

On March 24, 1882, Koch presented to the Physiological Society of Berlin a communication entitled "The etiology of tuberculosis", demonstrating the existence of *Mycobacterium tuberculosis* as the only causal agent of tuberculosis in all its forms, the route of subject-to-subject transmission, staining and culture methods, and forms of reactivation in humans^{10,16}.

With the stained bacillus in the patient's sputum, the laboratory begins the investigation of a sample of the patient's lesion in search of the germ, detects and evaluates the evolution of infectious cases, predicts and guarantees the cure of those who successfully complete the scheme and identifies those who fail with their treatment. The culture complements the staining of the germ since it allows to reveal viable bacilli present in small numbers in a lesion sample, to characterize them to certify that it is the tuberculosis bacillus and to know if it is sensitive or resistant to antituberculous drugs.

In the afternoon of March 24, 1882, at the Institute of Physiology of the University of Berlin, he gave a lecture in which he recounted how he had identified the bacillus in tuberculous material, its *in vitro* culture, and the production of the disease when he inoculated the pure culture in experimental animals, from whose tuberculous lesions he was able to isolate the bacillus again. When he finished his lecture, there was absolute silence in the room, everyone was amazed, the audience came to examine the microscopic preparations that he had brought. Paul Ehrlich, who would later improve the bacillus staining method, would remember that session for the rest of his life. The entire investigation took six months; his presentation, almost two hours, his projection, until today^{6,17}.

It is known that in this presentation he expressed the hope that the discovery would contribute to the development of effective measures to control the disease, attacking the causal agent or preventing its transmission.

The postulates raised by Robert Koch outlined a series of rules and requirements that must be followed when carrying out a study on the etiology of the disease, and which are stated in the following tenor: the same pathogen must be present in all cases of the disease; the pathogen must be isolated from the diseased host and grown as a pure culture; the pathogen from the pure culture must cause disease when inoculated into a healthy, susceptible animal; the pathogen must be isolated from the inoculated animal and must be shown to be the original microorganism².

When the scholar formulated his postulates, he was considered a visionary and a man ahead of his time, its application to microbiological studies allowed scientists to identify most of the pathogens that caused diseases with high mortality rates. Their importance lies in the fact that they mark a turning point in the history of microbiology by introducing the experimental method

for the first time. The postulates currently constitute the cornerstone of any study on the etiology of a disease, they allow the identification of new pathogens in order to apply preventive treatments. Researchers have managed to apply them in fields where it seemed impossible, such as biogeochemistry, bioremediation and the food industry¹¹.

However, the postulates have exceptions, situations in which they cannot be applied that is. It was Koch himself who, in the course of his research, recorded this, based mainly on the characteristics of both the bacteria and the disease it produces later.

His travels and research in Egypt, Africa and India are also considered significant, where he isolated and identified the bacillus that causes cholera, *Vibrio cholerae*, from samples obtained in the autopsy of patients^{9,11}.

In 1890, Koch exposed the results of research that he had carried out in secret, only in his laboratory: the discovery of a substance that could prevent the growth of the tubercle bacillus both in the test tube and in the body. Inoculation of it in guinea pigs made them resistant to the disease. He did not mention the nature and preparation of this substance, perhaps he was pressured by the German government to announce provisional results and achieve supremacy in microbiological research in the bitter struggle with France and Pasteur at the head. He also hastily experimented on man, at La Charité Hospital in Berlin. Most treated tuberculosis patients showed a strong hypersensitivity reaction to the inoculation, which was interpreted as a sign of cure and soon became known as Koch's phenomenon^{17,18}.

An individual who has not had contact with mycobacteria does not react to the test in which this substance is injected, a person who has had a primary infection with the bacterium reacts within hours by developing induration, edema, erythema, and in strongly positive reactions central necrosis.

Koch finally disclosed the nature of his remedy, which he called tuberculin, in 1891, a glycerinated extract of a pure culture of tubercle bacilli. Trials soon showed that it was not a curative remedy, but a diagnostic test. The treatment of tuberculosis with tuberculin announced in Berlin was considered a failure. However, Koch maintained until the end the belief in the curative value of tuberculin, and made several attempts to improve it^{17,18}.

Koch suffered criticism, when the therapeutic failure became evident, for his lack of scientific criteria, and for his incorrect behavior, when trying to keep the composition of the substance secret.

Between 1891 and 1904 he was director of the Institute for Infectious Diseases in Berlin, which currently bears his name. In 1905 he received the Nobel Prize for his research on tuberculosis. Robert Koch died on May 27, 1910 in Baden-Baden¹¹.

Louis Pasteur

Louis Pasteur was born on December 27, 1822, in the small town of Arbois in France. His parents were humble people, faithful to their religious beliefs and love of their country. Louis graduated with a bachelor's degree in 1840 from the Royal College of Bensaçon, Paris, studied at the École Normale Supérieure de Paris in the fields of Physics and Chemistry, and in 1847 obtained a Doctorate of Science. Due to his prestige already in 1857 he was appointed Director of Scientific Studies at the École Normale Supérieure de Paris, where he remained until 1863^{19,20}.

It is considered interesting the fact that despite not being a doctor, he came to exert a notable influence on his time with his research, which transcends to the present day, from the development that he fosters in branches of medicine such as microbiology and immunology.

Among his most significant contributions is the discovery of anaerobic germs when he studied butyric fermentation and introduced the concept of aerobic and anaerobic microorganisms. He demonstrated the erroneous nature of the spontaneous generation of germs and among his most outstanding achievements is the development of the controlled heating technique that is currently known as pasteurization, used by food companies in the preservation of beverages and substances¹⁹.

"Nature and the origin of fermentation", "Theory of fermentation" and "The pathogenic agents of contagious diseases" stand out as his first capital works. From his experimental studies with anthrax, he hypothesized that an infection occurred when a microorganism of a particular type was introduced into the body, confirming the cause-effect relationship of this theory with the use of a culture in serum and broth of the anthrax bacilli out of the infected body, showing that they retained the ability to cause the same infection in other healthy animals²⁰.

Throughout his life he followed the strictest rules of positivist thought and fought against any interpretation of biological phenomena based on unverifiable factors, such as the erroneous theory of spontaneous generation²⁰.

In 1880, when studying chicken cholera, he analyzed that aged cultures did not kill healthy chickens, but immunized them against virulent cultures, he related this to the Jennerian vaccination. From 1881 to 1884, he carried out vaccinations against anthrax, swine erysipelas, and rabies. These tests allowed him to develop the anthrax vaccine, whose famous experiment was carried out in Pouilly-le-Fort in 1881. Virulent strains of the anthrax bacillus were inoculated into forty-eight sheep and ten cows, of which twenty-four and six, respectively, had been previously vaccinated with inactivated preparations of the germ. All the vaccinated animals resisted the disease and all the non-vaccinated animals died³.

Thus began a brilliant career in immunology that would lead to products as amazing as the vaccine against chicken cholera, or even better against rabies, which was not even produced by bacteria but by a virus, a germ invisible to Pasteur and everyone else in that time.

Between 1880 and 1885 he developed the study and application of the rabies vaccine; in July 1885, he decided to apply the treatment to the young Joseph Meister, bitten by a rabid dog; Dr. Grancher gave the boy thirteen inoculations of rabid rabbit marrow emulsion. The second case was that of Pastor Jupille. On October 26, 1885, he informed the Academy of Sciences of the success of these two treatments and a year later, almost two thousand five hundred people had been vaccinated³.

In 1887, he discovered that environmental bacteria could destroy *Bacillus anthracis* and that animals infected with other microorganisms were resistant to anthrax. This interference phenomenon was called antibiosis and it would help the study and understanding of antimicrobial mechanisms. Due to his multiple investigations, he was elected a member of the French Academy of Medicine, an honor that was usually only granted to doctors of high merit^{12,19}.

The authors consider that the interest of the scholar in finding in microorganisms the origin of most of the pathophysiological processes to seek, the means to put an end to the biological balance disorder; definitively fixes the doctrine of operative realism that prevails in life sciences.

The success of the vaccine caused the government to issue a universal subscription or collection law for the construction of the Institute that would bear his name. On November 14, 1888, the Pasteur Institute in Paris was inaugurated, creating other institutes with the same name in the world. They became hubs of the new science, with doctors and researchers from all countries. His disciples also stood out as recognized teachers in the areas in which they worked, examples of which were Metchnikoff, Roux, Yersin and Calmett^{3,19}.

He bequeathed to humanity the practice of vaccination, since then vaccines are a very important part of preventive therapy in dissimilar diseases¹⁰.

Many germs responsible for infectious diseases were discovered, not only due to the work of Pasteur but also of Koch, between the end of the 19th century and the beginning of the 20th century, where the isolation of the causal agents of gonorrhoea stands out (Neisser, 1879), diphtheria (Klebs, 1883 and Loeffler, 1884), tetanus (Nicolaier, 1885 and Kitasato, 1889), pneumonia (Fraenkel, 1886), meningitis (Weichelbaun, 1887), bubonic plague (Yersin, 1894), leishmaniasis (Leishman and Donovan, 1903), syphilis (Schaudinn and Hoffman, 1905), and Rocky Mountain spotted fever (Ricketts, 1909), which would also have been impossible without the useful microscope^{20,21}.

Pasteur was director of the Institute that bears his name until his death and in 1874 the National Assembly awarded him a high life pension. The Academy of Medicine welcomed him in its bosom in 1881 and nine years later the Sorbonne paid him homage, which was attended by the President of the Republic^{19,20}.

His work was vital for the English surgeon Joseph Lister, who immediately understood the value of the discovery of inhibition, created by antiseptics in the growth of microorganisms in culture media, and created antiseptics with immersion of all surgical instruments before a surgery in phenolic acid and washing hands with a detergent, and with it the infections of the surgeries decreased remarkably²².

The discovery and understanding of the existence of numerous substances with toxic effects for bacteria, but innocuous for man, would constitute a pending issue for posterity, which would guide pharmacological treatments for the benefit of humanity.

Pasteur died on September 28, 1895, at his home in Garches-Villeneuve-l'Étang, on the outskirts of Paris²⁰.

Despite the fact that chronic non-communicable diseases are currently important causes of mortality worldwide, infectious processes continue to occupy top places. There are projections that suggest that more people will die from infections than from cancer, which is why adequate resources and policies are required to face and counteract this reality²³.

In modern medical microbiology, rapid and effective microbiological diagnosis is advocated, which allows specific treatment for patients, so that as soon as possible they stop being contagious for the components of their social group, thus preventing the appearance and spread of outbreaks and epidemics.

CONCLUSIONS

The formulation of the germ theory or germ theory of disease is the culmination of the work carried out by Louis Pasteur and Robert Koch, the first on the fermentation of wine and beer; and the second on anthrax and tuberculosis. This theory broke with old schemes, it was based on experimental observation and opened the era of the modern concept of causality, supported by the attributes of association, temporality and direction.

AUTHORSHIP

Rita María Sánchez-Lera: conceptualization, research, methodology, validation-verification, writing-original draft, writing-review and editing.

Isael Armando Pérez-Vázquez: formal analysis, project management, research, resources, writing-original draft, writing-review and editing.

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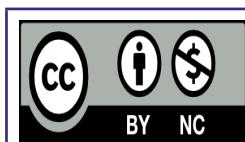
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Pasteur y Koch: los padres de la microbiología

RESUMEN

Introducción: los científicos Louis Pasteur y Robert Koch realizaron trascendentales contribuciones a la medicina, a la ciencia en general, y en particular a la microbiología, muchas de las cuales transmiten un conocimiento irrefutable y elevado. **Objetivo:** describir la labor realizada por Pasteur y Koch en el campo de la microbiología. **Método:** se realizó una revisión bibliográfica en el periodo de noviembre a diciembre de 2020 utilizando como recursos de información los disponibles a través de la red Infomed y las bases de datos PubMed, SciELO y LILACS. Los términos empleados en la búsqueda fueron: "Bacteriología", "Historia de la medicina" y "Tuberculosis"; se conformó la siguiente estrategia de búsqueda: [(Bacteriología) AND (Historia de la medicina) AND (Tuberculosis)]. **Desarrollo:** los aportes de estos grandes de la ciencia marcaron un antes y después en el campo de la microbiología; con sus descubrimientos establecieron postulados y teorías que con posterioridad constituirían las bases para el estudio de los agentes patógenos causales de las enfermedades. A partir de esto fue posible el desarrollo de terapias preventivas puesto que se conocían en su mayoría los agentes causales. **Conclusiones:** la formulación de la teoría del germen o teoría microbiana de la enfermedad fue el mayor discernimiento en las investigaciones de ambos..

Palabras clave: Bacteriología; Historia de la Medicina; Tuberculosis.



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