Review Article

Zhuoyang Zhang and Wei Zhao*

Koch's postulates: from classical framework to modern applications in medical microbiology

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Abstract: Koch's postulates, formulated by Robert Koch in the late 19th century, remain a foundational pillar of medical microbiology. They provide a systematic framework for establishing causal relationships between pathogens and diseases. While the original postulates revolutionized infectious disease research, their limitations in addressing the complexities of modern microbiology have led to necessary adaptations. The article systematically revisits the classic framework of Koch's postulates and highlights its expansion and adaptation in light of modern technologies such as molecular biology, genomics, and microbiomics. By integrating frameworks such as One Health and reverse microbial etiology, Koch's postulates continue to play a critical role in addressing global health challenges. Furthermore, the article emphasizes the significance of Koch's postulates in medical education, noting the role as a core tool for scientific reasoning and diagnostic logic training, equipping medical students to tackle modern challenges like antibiotic resistance and novel pathogen.

Keywords: Koch's postulates; medical microbiology; pathogen identification

Koch's postulates: the primitive framework for pathogen identification

Proposed by the German physician and microbiologist Robert Koch (1843–1910) in the late 19th century, Koch's postulates establish a set of criteria to determine whether a specific microorganism serves as the causative agent of a particular disease. The classic Koch's postulates consist of the following four principles:

(1) Specific pathogens should be detected in patients with the same disease but not in healthy individuals. That is, a particular pathogen should be present only in disease-associated patients and absent in healthy ones.
(2) Special pathogenic bacteria can be isolated and cultured from the patient's body to obtain pure strains. In other words, pathogenic bacteria should be isolatable from the patient's body and culturable under laboratory conditions.
(3) This pure culture can be inoculated into susceptible animals, resulting in the same symptoms. Namely, inoculating the pure culture into susceptible subjects should induce the same symptoms as observed in humans.
(4) The pure culture of the pathogen can be re-isolated from artificially infected experimental animals to verify its pathogenicity.

The formulation of these postulates marked the foundation of modern medical microbiology. They clarified the causal relationship between pathogens and diseases, providing critical guidance in identifying new pathogens [1]. With the advent of Koch's postulates, the development of pathogenic microbiology accelerated. This principle is applicable not only to bacteria but also widely utilized in the study of other pathogens such as viruses and parasites. By means of Koch's postulates, more accurate disease diagnosis can be achieved, and effective treatment plans can be developed for specific pathogens. It also plays a significant role in the prevention and control of infectious diseases.

The technological revolution has propelled human civilization to unprecedented heights while accelerating the advancement of modern medicine. The proposed new concept in healthcare represents a strategic transition from a treatment-centered approach to a comprehensive life-cycle medical model that integrates prevention, treatment, rehabilitation, and health maintenance, establishing a holistic framework for whole-person care throughout the human

***Corresponding author: Wei Zhao**, Center for Experimental Medical Science Education, Shanghai Jiaotong University School of Medicine, Shanghai 200025, China, E-mail: zhaowei_sjtu@sjtu.edu.cn
Zhuoyang Zhang, Center for Experimental Medical Science Education, Shanghai Jiaotong University School of Medicine, Shanghai, China.
https://orcid.org/0000-0003-4283-521X

lifespan. Under this transformative background, the seamless integration of traditional medical concepts into the teaching of New Medicine (NM), which integrates advanced technology and interdisciplinary approaches to transform healthcare through innovation and precision [2], poses a challenge for medical educators. This integration challenge becomes particularly evident in reconciling Koch's postulates with the NM paradigm:

(1) The preventive life-cycle model challenges Koch's "one pathogen, one disease" framework because the immune status, physiology, and genetic variability of the host play crucial roles in disease manifestation.
(2) Genomic technologies like metagenomic sequencing, now central to NM, transcend Koch's requirement for pathogen isolation, enabling pre-symptomatic pathogen detection through environmental microbiome monitoring.
(3) The recognition of asymptomatic carriers (e.g., *Helicobacter pylori* colonization in healthy individuals) fundamentally challenges Koch's first postulate regarding pathogen-free healthy hosts.

Notably, the emerging "reverse microbial etiology" concept exemplifies this paradigm shift, proactively establishing pathogen warning systems through environmental surveillance rather than post-outbreak pathogen pursuit.

As the most important fundamental principle of microbiology, Koch's postulates are an essential part of every medical student's learning process. This framework not only helps medical students develop critical scientific reasoning but also equips them to establish precise diagnostic approaches and optimize therapeutic interventions in their clinical practice. Through critical analysis of how Koch's postulates have evolved and where they fall short, medical students develop critical thinking skills to apply this core principle to modern diagnostic tools and antibiotic resistance challenges. Therefore, Koch's postulates remain indispensable in medical education.

Challenges and modern revisions of Koch's postulates

Limitations of the classic Koch's postulates

Although Koch's postulates are of great guiding significance in identifying new pathogens, their limitations have gradually become apparent in practical applications, making it difficult to apply them to various special cases and emerging diseases. These limitations render Koch's postulates not universally applicable in certain situations. The limitations are primarily manifested in the following aspects [1, 3]:

(1) The presence of carriers may lead to different infection outcomes with the same pathogen due to the host's immune status. Pathogens can survive and spread in asymptomatic carriers, so many pathogens can also be isolated from healthy individuals, such as *Candida albicans* and *Neisseria meningitidis.*
(2) Certain pathogens (e.g., *Staphylococcus aureus*) induce disease via toxin production or immune-mediated damage, rendering them non-culturable during early infection stages, as exemplified by toxic shock syndrome.
(3) Not all microorganisms can be cultured under laboratory conditions, such as *Mycobacterium leprae.*
(4) Some pathogens can only infect specific hosts, and it is difficult to find suitable animal models, such as herpes simplex virus.
(5) The occurrence of many diseases is the result of multiple factors working together, including the host's genetic susceptibility, environmental factors, and microbial factors, such as co-infection of hepatitis D virus and hepatitis B virus.

These limitations of classical postulates have catalyzed the integration of molecular and genomic innovations. From Rivers' virological adaptations to Falkow's gene-centric criteria, and ultimately to genome-wide analyses, each advancement addressed specific gaps in pathogen identification – whether by bypassing cultivation barriers (e.g., *M. leprae*) or resolving multi-factoral disease mechanisms (e.g., hepatitis co-infections). Collectively, they represent a continuum of methodological evolution, driven by technological progress and expanding biological complexity.

Modern revision of Koch's postulates

With the development of science and technology, especially the progress of genomics and molecular biology techniques, scientists trace the origin of infectious diseases with the rigorous spirit of Koch's postulates and apply new methods to obtain scientific evidence to prove the pathogen of infectious diseases, giving Koch's postulates a new interpretation and application. Many scholars constantly propose supplementary viewpoints and attempt to improve Koch's postulates, expanding the relevant knowledge of this classic principle and enriching its content.

In 1937, Thomas Rivers, an American virologist, proposed modified guidelines for viral diseases [1]. Unlike the classical Koch's postulates, Rivers' modifications consider the presence of healthy carriers, adjust the first rule of the

classical postulates, and waive the requirement for reproduction in culture medium or cell culture.

With the purification of viral antigens and the establishment of specific antibody detection methods, immunological methods have been widely used in the study of microbial etiology. Building upon Koch's postulates, Alfred Evans introduced expanded immunological and epidemiological criteria for causal inference [4]. His framework extended the principles of disease causation to account for multifactoral and even non-infectious diseases, addressing the limitations of traditional postulates.

With the discovery of an increasing number of viruses, some scholars have proposed various revisions for chronic infections, latent infections, multiple viral infections, viral immunity, viral tumorigenicity, lentiviruses, prions, etc.

While Rivers' modifications addressed viral detection challenges in asymptomatic carriers, the rise of molecular biology demanded a more granular approach. Stanley Falkow's molecular Koch's postulates (1988) [5] extended this framework by linking pathogenicity to specific virulence genes, thereby enabling functional validation through genetic manipulation – a paradigm shift from observational to mechanistic evidence. Stanley Falkow believed that the inherent logic of Koch's postulates could be applied to the association between microbial genes and pathogenicity.

In 1996, David Fredricks and David Relman proposed nucleic acid-based revisions to Koch's postulates, emphasizing causal inference through sequence evidence rather than strict adherence to classical criteria [6]. Their framework requires that a pathogen's nucleic acid be predominantly detected in diseased tissues, correlate with disease severity or recovery, and align with the biological traits of related microorganisms. The copy number of the pathogen's sequence should also show dynamic changes mirroring clinical progression (e.g., decreasing upon recovery or rising during relapse). Additionally, cellular-level evidence must demonstrate pathogen-tissue interaction, with findings being reproducible across studies.

Challenges in complex pathogen-host interactions

The classical Koch's postulates struggle to address the host-dependent nature of conditional pathogens, where disease manifestation hinges on specific host vulnerabilities rather than intrinsic microbial pathogenicity alone. For instance, commensal *Escherichia coli* strains typically reside harmlessly in the intestinal lumen but can cause severe infections (e.g., bacteremia) when host defenses are compromised, such as during immunosuppression or intestinal barrier dysfunction [7]. Genomic analyses demonstrate that host-derived signals – like pro-inflammatory cytokines during immunosuppression – activate otherwise silent virulence genes (e.g., adhesin genes and invasin genes) in these commensal bacteria, enabling tissue invasion and systemic spread [8]. This interplay highlights that pathogenicity is not an absolute microbial trait but a dynamic outcome of host-microbe interaction. Consequently, modern pathogen identification must integrate assessments of host status (e.g., immune competence, epithelial integrity) alongside microbial genomic profiling to establish causality in infections involving conditional pathogens.

Polymicrobial infections further challenge single-pathogen causality. Periodontitis, for instance, involves synergistic interactions between *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* [9]. The "red complex" in endodontic infections, made up of the three bacteria, has multiple pathogenic mechanisms. Each bacterium has its own virulence factors and they show synergistic effects, like nutritional interactions. They disrupt the host's immune response, causing persistent inflammation. Additionally, they can form biofilms, which protect the bacteria and make infections more difficult to treat. Such microbial consortia evade traditional postulates, as no single organism fulfills all criteria independently.

Alternative model innovation

Ethical constraints on human experimentation have driven innovations in alternative models. For example, SARS-CoV-2-infected human angiotensin-converting enzyme 2 (hACE2) mice develop acute respiratory distress, validating the virus's causative role in COVID-19 without direct human trials [10]. Similarly, *in vitro* organoid models of intestinal or lung tissue enable mechanistic studies of pathogen invasion (e.g., *Cryptosporidium trophozoite* penetration [11]) and host-cell remodeling under controlled conditions [12]. These approaches not only address ethical concerns but also provide granular insights into host-pathogen dynamics that are unattainable through classical methods.

By integrating genomic tools and ethical alternatives, modern microbiology navigates the limitations of Koch's framework while preserving its core emphasis on causal evidence [13].

The modern application of Koch's postulates

The limitations of Koch's postulates have long been recognized, particularly in their inability to address certain complexities of modern microbiology and

Table 1: Limitations of classical Koch's postulates and corresponding molecular/genomic solutions.

	Specific pathogen or disease	Molecular/genomic solutions
Cannot detect asymptomatic carriers	**Hepatitis B virus (HBV)**: some carriers are asymptomatic but can transmit the virus	**PCR and sequencing**: detect HBV DNA in asymptomatic carriers
Polymicrobial infections	**Periodontal disease**: caused by multiple bacteria (e.g., *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*)	**Metagenomics**: identify and quantify microbial communities associated with periodontal disease
Difficult to cultivate or non-culturable microbes	***Treponema pallidum* (causative agent of syphilis):** difficult to culture *in vitro*	**Cell culture and co-culture systems**: use host cells to grow *Chlamydia* **Genome sequencing and metagenomics**: directly analyze the genetic material of *Treponema pallidum*
Lack of suitable animal models	**Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)**: infects humans but lacks suitable animal models	**Alternative animal models**: transgenic murine models for investigating COVID-19 pathogenesis
Host-specific responses	**Human immunodeficiency virus (HIV)**: infects humans but not other primates (e.g., chimpanzees)	**Animal models and CRISPR/Cas9**: study host-specific genes (e.g., C–C chemokine receptor 5 [*CCR5*]) in HIV infection
Environmental factors	**Legionnaires' disease**: caused by *Legionella*, which thrives in warm and humid environments	**Transcriptomics and proteomics**: study gene expression and protein profiles of *Legionella* under different environmental conditions

infectious diseases. With the development and application of advanced technologies such as molecular biology, genomics, and metabolomics, these limitations have been effectively addressed (Table 1). These innovations have progressively enhanced the ability to study and understand infectious diseases, overcoming the constraints of traditional methods. By leveraging high-throughput sequencing, bioinformatics, and systems-level analyses, a more comprehensive framework for pathogen research has emerged. This modern framework integrates genomic sequence analysis, comparative genomics, host-pathogen interaction studies, and environmental genomics, providing a holistic approach to pathogen identification, virulence assessment, and disease control. Thus, Koch's postulates have evolved into a robust and adaptable guideline for contemporary microbiology, enabling more precise and efficient research in infectious diseases.

Genomic adaptations of Koch's postulates

Genomics has gradually become an important tool for life science research. It not only provides genetic information about species, but also reveals gene expression regulation, evolutionary relationships, and interactions between pathogens and hosts. Genomic adaptations of Koch's postulates leverage high-throughput sequencing and bioinformatics to enhance pathogen identification. The genomic Koch's postulates are an extension and supplement to the traditional Koch's postulates, mainly including the following aspects:

(1) Genomic sequence analysis: By sequencing and analyzing the genomes of pathogens, the types and subtypes of pathogens can be more accurately identified. Genomic data can also reveal virulence factors, resistance genes, and potential pathogenic mechanisms of pathogens.

(2) Comparative genomics: By comparing the genomes of different pathogens, the evolutionary relationships and functional differences between them can be revealed. This is of great significance for understanding the evolutionary history and transmission pathways of pathogens.

(3) Host pathogen interaction research: Genomic techniques can be used to study the interactions between pathogens and hosts, including host immune responses and mechanisms by which pathogens evade the immune system. This helps to develop new treatment strategies and vaccines.

(4) Environmental genomics: By analyzing the microbial community genome in the environment, we can understand the distribution and dynamic changes of pathogens in the environment, thereby better preventing and controlling infectious diseases.

(5) Functional genomics: Using gene knockout, overexpression and other methods to study the function of pathogenic genes can reveal their specific mechanisms of action and provide a basis for the discovery of drug targets.

The genomic Koch's postulates have significant advantages over the classical:

(1) Fastness: High throughput sequencing technology can obtain a large amount of genomic data in a short period of time, greatly reducing the time required for pathogen identification.
(2) Accuracy: Genomic sequence-based identification methods are more accurate than traditional phenotype-based methods and can distinguish between different species and strains of pathogens.
(3) Comprehensiveness: The genomic Koch's postulates not only focus on the pathogenicity of pathogens but also on their drug resistance, evolutionary relationships, and other aspects, providing more comprehensive information for the prevention, control, and treatment of pathogens.

During the COVID-19 pandemic, the discovery of SARS-CoV-2 represented a successful integration of Koch's postulates and genomics. Initially, viral RNA was isolated from patient samples and sequenced, confirming its classification within the β-coronavirus genus, thereby fulfilling the first postulate of Koch that a specific pathogen must be present in diseased individuals [14, 15]. Subsequently, experimental evidence demonstrated that the spike protein (S protein) of SARS-CoV-2 could bind to the hACE2 receptor, endowing it with the capacity to infect human epithelial cells. Further validation was achieved through the use of a transgenic mouse model expressing hACE2, which reproduced COVID-19-like pulmonary pathology upon SARS-CoV-2 infection and allowed for the re-isolation of the virus, thus completing the application of Koch's postulates [16]. This process exemplifies the flexible application of Koch's postulates in modern pathogen research, involving virus isolation, molecular mechanism elucidation, pseudovirus experiments, and animal modeling to establish the causative relationship between SARS-CoV-2 and COVID-19. Despite certain modifications due to technical limitations, the core logic – association between pathogen and disease, transmissibility, and re-isolation for validation – remains a cornerstone in etiological studies. Similar applications have also been observed in research related to Mpox and avian influenza outbreaks.

Microbiome adaptations of Koch's postulates

With the development of modern science and technology, microbiome technology has provided new perspectives and tools for Koch's postulates, enabling us to gain a deeper understanding of the interactions between microorganisms and their hosts, as well as their impact on health and disease.

The development of microbiome technology has provided new tools and methods for studying microbial communities. Through high-throughput sequencing technology, scientists can reveal the structure and function of microbial communities in complex environments, as well as their interactions with hosts and the environment. These studies not only cover ecosystems such as soil, ocean, and glaciers, but also include host related microbial communities such as humans, plants, and animals.

The relationship between the microbiome and host health is increasingly being emphasized. Research has shown that imbalances in the human microbiome are associated with the occurrence and development of various diseases. The gut microbiota can maintain a stable relationship under disturbances such as dietary changes and disease states, forming two competitive guilds (TCGs), one beneficial to health and the other harmful, affecting human health like a seesaw [17]. This discovery provides a microbiome based diagnostic and therapeutic strategy that can deepen the understanding of microbiome function by identifying core microbiota, providing living biomarkers and therapeutic targets for precision medicine. It can also exert protective effects on the host by regulating the composition and function of the microbiome. For instance, modulating the composition and functionality of the microbiome – through interventions such as natural or synthetic microbial consortia – enables regulation of the host's immune responses and metabolic pathways.

Koch's postulates and microbiome have close connections and complementarity in microbiological research. Koch's postulates provide a foundation for the study of the microbiome, especially in the identification of pathogenic microorganisms and their pathogenic mechanisms. The study of the microbiome has driven the development of Koch's postulates, providing new methods for pathogen detection and identification through more advanced technological means such as gene sequencing, gene editing, and immunological methods. The integration of Koch's postulates with the microbiome has led to the notion that diseases are no longer attributed to a single pathogen. Instead, the microbiome is regarded as a dynamic ecosystem, in which the overall state or key functional modules play a causal role in complex diseases. This provides theoretical support for precision medicine and ecological therapies, such as microbiome-targeted therapies [18].

Liping Zhao used Evans's modified version of Koch's postulates as a conceptual framework for organizing evidence to demonstrate the causative role of the gut

microbiota in obesity [19]. His research revealed that one endotoxin-producing bacterium (*Enterobacter cloacae* B29) isolated from a morbidly obese human's gut induced obesity and insulin resistance in germfree mice [20].

Fusobacterium nucleatum, an opportunistic anaerobic bacterium naturally found in the oral microbiota, has been identified as a dominant species in the colons of patients with colorectal cancer (CRC). *F. nucleatum* produces the virulence factor 3-ketoacyl-CoA thiolase FadA (FadA), which enhances the permeability of colonic epithelial cells. Studies have shown that treating *Fusobacterium*-positive colon cancers with antibiotics leads to a reduction in tumor growth, bacterial load, and cancer cell proliferation [21]. These findings strongly support the association of *F. nucleatum* with cancer progression, highlighting its potential role as a driver of CRC development [22].

Combination of Koch's postulates with One Health and reverse microbial etiology

The One Health framework [23], emphasizing interconnected human–animal–environment health systems, provides the ideal platform for unifying Koch's postulates with reverse microbial etiology. This triad bridges traditional pathogen confirmation, proactive surveillance, and holistic ecosystem management. Reverse microbial etiology – proposed by Professor Jianguo Xu in 2019 – advocates preemptive identification and evaluation of potential pathogens through genomic technologies to prevent outbreaks [24]. The proposal of reverse microbial etiology marks a shift from passive response to infectious diseases to active exploration and prevention, early detection and evaluation of potential pathogens, in order to prevent possible future outbreaks of infectious diseases. Its objectives align with One Health by addressing cross-sectoral risks and fostering interdisciplinary collaboration among public health, veterinary, and environmental sectors, providing new avenues for the prevention and control of emerging infectious diseases.

There are two concepts "reverse zoonosis" and "pathogen discovery pipelines," that are similar to the concept of reverse microbial etiology. The concept of reverse microbial etiology shares strategic similarities with Western frameworks like pathogen discovery pipelines while maintaining distinctive theoretical orientations. The PREDICT program of the Centers for Disease Control and Prevention (CDC) of the United States employed metagenomic surveillance to identify novel viruses in wildlife reservoirs, aligning with reverse microbial etiology's proactive screening philosophy [25]. Both approaches utilize high-throughput sequencing to build pathogen databases for outbreak preparedness. However, reverse microbial etiology uniquely emphasizes cross-species transmission risk prediction through evolutionary genomics, whereas pathogen discovery pipelines focus primarily on cataloging microbial diversity.

Regarding reverse zoonosis (human-to-animal transmission), reverse microbial etiology provides complementary insights [26]. While reverse zoonosis studies pathogen spillback mechanisms (e.g., SARS-CoV-2 transmission to minks), reverse microbial etiology systematically assesses bidirectional risks at the human–animal–environment interface through genomic virulence markers and host tropism analysis. This framework enhances predictive capability for both classic zoonoses and emerging reverse zoonotic events.

The integration of Koch's postulates with reverse microbial etiology and One Health bridges classical pathogen confirmation with proactive pathogen surveillance. While Koch's postulates traditionally require isolating pathogens after disease manifestation, reverse microbial etiology flips this paradigm by employing metagenomic sequencing to catalog environmental microorganisms before outbreaks occur. This approach addresses three key limitations of classical postulates:

(1) Pre-symptomatic detection: By analyzing microbial communities in environmental reservoirs (e.g., wastewater [27] and wildlife [28, 29]), potential pathogens can be identified before human transmission, circumventing Koch's requirement for symptomatic hosts. In 2010, Yongzhen Zhang's research team reported the discovery and characterization of a tick-borne virus, Jingmen tick virus (JMTV), in ticks samples [30]. Four JMTV-infected patients were identified by high-throughput sequencing of skin biopsies and blood samples in 2019 [31].
(2) Non-culturable pathogens: Many microbial species resist traditional culturing methods. Metatranscriptomic analysis enables functional assessment of these non-culturable organisms, fulfilling Koch's second postulate through genomic proxies rather than pure isolates [32].
(3) Ecological context: Koch's postulates focus on host-pathogen dyads, whereas reverse microbial etiology incorporates ecosystem-level interactions aligned with the One Health framework, revealing how environmental changes potentiate pathogen emergence.

This synergy is exemplified in pandemic preparedness: Jing Wang et al. [33] characterized the mammal-associated viruses in 149 individual bats sampled from Yunnan

province, China, using an unbiased metatranscriptomics approach. Through phylogenetic analysis, five viral species closely related to known human or livestock pathogens were identified, including a novel recombinant SARS-like coronavirus. The identification of potentially pathogenic viruses to humans and livestock, especially the novel recombinant SARS-like coronavirus, emphasizes the importance of continuous surveillance of bat populations.

The integration of Koch's postulates, reverse microbial etiology, and One Health creates a robust paradigm for 21st-century health security. The rigor of Koch's postulates in causality establishes diagnostic certainty, reverse microbial etiology enables preemptive threat detection, and One Health ensures these efforts address interconnected human–animal–environment systems. This triad transforms infectious disease management from reactive containment to proactive prevention, epitomizing the adage: "Prevention is better than cure." As climate change and globalization intensify zoonotic risks, this synergy will be pivotal in safeguarding global health.

The significance of Koch's postulates

Koch's postulates, originally designed to establish causality between pathogens and diseases, have been modernized through advancements in molecular biology and genomics, providing a robust framework not only for guiding public health strategies but also for underscoring significant philosophical implications in understanding disease mechanisms.

Scientific basis for public health decision-making

Epidemic tracing

Pathogen gene sequencing has become an indispensable tool for tracing transmission chains and understanding outbreak dynamics. For example, since the H5N1 avian influenza outbreak, comparative genomic analysis of viral isolates from different species and geographic regions has revealed critical insights into the virus's cross-species transmission pathways [34]. This approach aligns with Koch's postulates by confirming the presence of the pathogen in infected hosts and establishing its role in disease spread. Similarly, during the COVID-19 pandemic, whole-genome sequencing of SARS-CoV-2 variants enabled global surveillance of viral evolution and transmission patterns, guiding public health responses [35].

Antimicrobial resistance monitoring

The dissemination of resistance genes, such as the carbapenemase gene, subclass B1 metallo-beta-lactamase NDM-1, has been tracked using genomic methods, providing a scientific basis for combating drug-resistant infections. Genome sequencing of bacterial isolates from clinical and environmental samples has revealed the global spread of resistance genes, offering insights into their transmission dynamics and mechanisms [36]. This approach adheres to Koch's logical framework by establishing a causal link between the presence of resistance genes and the emergence of multidrug-resistant pathogens, informing strategies for containment and treatment.

Philosophical significance

Beyond its practical applications, Koch's postulates hold profound philosophical significance in modern medical research, emphasizing the establishment of causality rather than mere correlation.

From correlation to causation

Koch's postulates help avoid misclassifying commensal microorganisms as pathogens, a critical issue in the era of microbiome research. For instance, the complex relationship between gut microbiota and conditions like obesity, diabetes, and inflammatory bowel disease underscores the importance of distinguishing between association and causation. By applying Koch's principles, researchers can identify true pathogenic microbes and develop targeted interventions, avoiding the pitfalls of overgeneralization [19].

From single factors to multi-factoral networks

Koch's principles have evolved to integrate host genetics, immune status, and microbial ecology, reflecting the multifactoral nature of infectious diseases. For example, the interplay between host genetic polymorphisms (e.g., in the ACE2 receptor) and viral pathogenicity in COVID-19 highlights the need for a systems-level approach [37]. Similarly, the role of immune dysregulation in chronic infections like tuberculosis [38] demonstrates how Koch's framework can be expanded to include host-pathogen interaction networks, enabling a more comprehensive understanding of disease mechanisms.

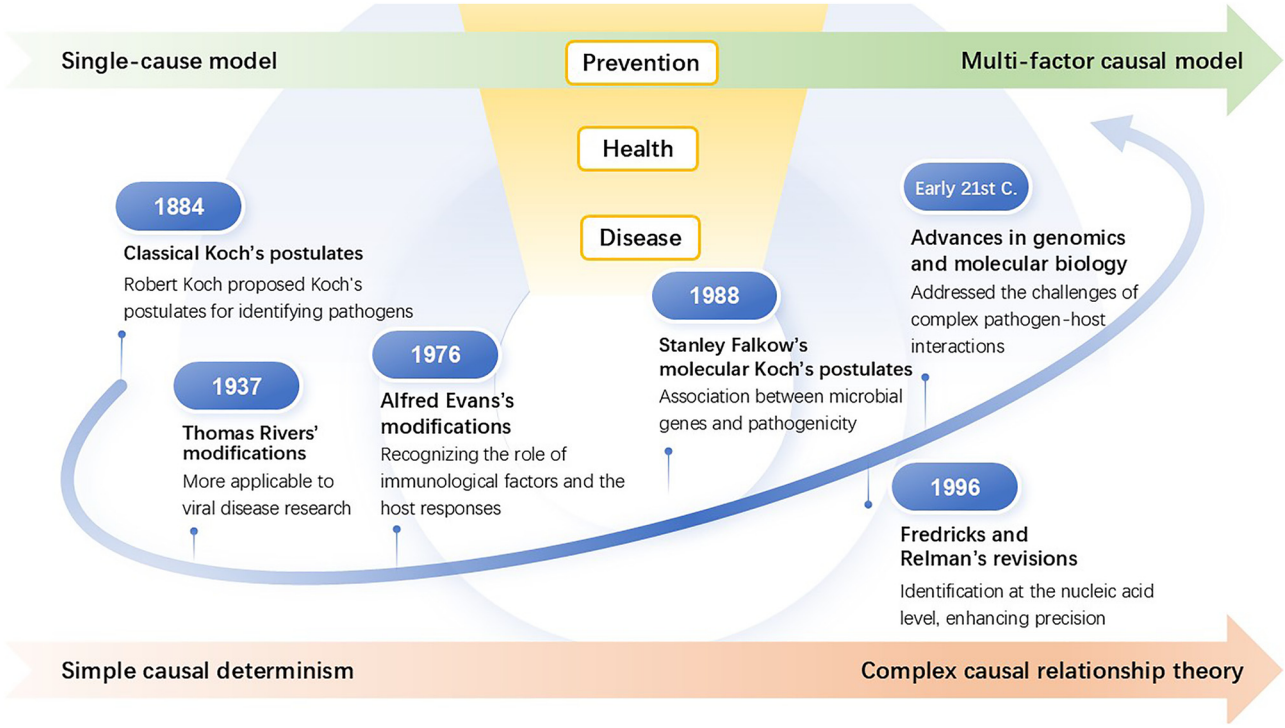

Figure 1: The evolution of Koch's postulates.

Conclusions

As the "first principle" of medical microbiology, Koch's postulates have been evolving and persistently underscored the causal link between pathogens and diseases, with their enduring value rooted in three core contributions: (1) providing a systematic logical framework for pathogen identification; (2) standardizing research methodologies for elucidating pathogenic mechanisms; and (3) guiding the targeted development of diagnostic, therapeutic, and preventive strategies. While modern technologies – such as molecular biology and genomics – have vastly expanded their applications, the foundational tenet that "definitive pathogen identification remains the cornerstone of infectious disease research" remains immutable (Figure 1).

The adaptability of Koch's postulates to contemporary scientific advancements ensures their sustained relevance. Similarly, their integration into the One Health paradigm demonstrates their utility in addressing zoonotic threats, where establishing transmission pathways between environmental reservoirs and humans is critical. This holistic approach is indispensable for tackling pandemics and climate-sensitive health crises.

Looking forward, Koch's postulates will continue to evolve alongside emerging technologies to combat antimicrobial resistance and novel pathogens. Medical research must transcend a disease-centric model, embracing interdisciplinary collaboration and systems-level analyses aligned with One Health principles. By preserving the rigorous scientific ethos embedded in Koch's framework while innovating through genomic, microbiome, and AI-driven tools, future efforts will revolutionize pathogen detection, therapeutic design, and ecological health. Ultimately, this dynamic synergy between classical principles and modern methodologies will drive transformative advances in global health resilience.

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