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REVIEW ARTICLE

Koch Postulate: Why do we Should Grow Bacteria?

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Understanding infectious diseases has long relied on the Koch postulate, which consists of the pure culture of microorganisms. The advent of molecular methods in clinical microbiology has led the phasing out of culture as a diagnostic tool and metagenomics has become the technique most commonly used to assess the impact of commensal microbes on human health. However, culturing microbes has led to substantial advances, even recently, in infectious diseases involving fastidious microorganisms, as evidenced by the *Tropheryma whipplei or Bartonella* species. This allows their genomes to be sequenced and, consequently, new diagnostic tools to be acquired, experimental model to be created and antibiotic susceptibility testing to be performed. In addition, extensive culture focused on isolation of human commensals, know as the culturomics approach, has increased the number of bacteria isolated from humans by more than 35% over the past five years. As strains belonging to the same species can have different impacts on human health, it would appear necessary to pursue efforts to constitute an exhaustive bank of isolates in order to establish further proof of concepts. This review discusses recent examples, including the influence of *Akkermansia muciniphila* and *Faecalibacterium prausnitzii,* as well as the secretion of lugdunin by *Staphylococcus lugdunensis* which is efficient against the nasal carriage of *Staphylococcus aureus*. Finally, responses to some anti-cancer therapies and the treatment of *Clostridium difficile* infections through the use of fecal microbiota transplantation clearly suggest that culturing marks the beginning of bacteriotherapy.

Introduction

The pure culture of microorganisms has long been considered as a fundamental step in microbiological research (1). From Pasteur's work on fermentation in 1861 through to the introduction of molecular methods at the end of twentieth century, culture has been the only direct technique used to establish a link between microorganisms and human diseases (1). In the past, clinical microbiologists and scientists focused their research exclusively on microorganisms considered as pathogenic for humans. With the advent of molecular tools over the past 25 years, some authors have proposed that using 16S rRNA amplification and sequencing (2) and, more recently, using metagenomics (3), could replace the culture of microorganisms.

Understanding infectious diseases has long relied on Koch postulates which consists of the pure culture of microorganisms (4). Unfortunately, culturing has been progressively abandoned by most clinical microbiology laboratories and is currently confined to some microbiological examinations, such as bacteremia or UTI etiological diagnosis, while fastidious microorganisms are preferentially detected using molecular methods. Crucially, environmental microbiologists re-introduced culturing techniques (5−8). Over the past five years, microbial culturomics, a high throughput culture method multiplying the number of culture conditions and using a rapid identification method by MALDI-TOF mass spectrometry (9−11), has been developed to study the human gut microbiota. Consequently, culturomics has doubled the number of known bacteria cultured from human gut (11). Simultaneously, the relationship between human microbiota and various diseases has become a hot topic. This explains why interest in commensal microorganisms has dramatically increased in recent years.

In this paper, we propose a mini-review highlighting the need to go beyond Koch's postulate through recent changes in clinical microbiology including the study of human microbiota and culturomics.

Koch Postulate

In 1890, Robert Koch formulated postulates defining the criteria required to incriminate a bug (firstly a parasite) as the causative agent of infectious diseases (4), for which

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he was awarded the Nobel Prize for Medicine in 1905. His theory was sustained by the formulation: 1 pathogen + 1 host = 1 disease. First, the microorganism should be encountered in all the cases of the diseases. Secondly, this microorganism should not occur as commensal in healthy individuals. Finally, the causative agent can be isolated from one patient and, after several propagations in pure culture, can further cause this disease when re-inoculated (12,13).

One of the most famous examples of the Koch postulate was provided by Marshall who, after five days' incubation, identified small colonies from the stomach of patients suffering from gastritis (14). Because the causative relationship between this bacterium and gastritis was challenged by the international scientific community, Marshall demonstrated the Koch postulate., The bacteria was orally administrated to a volunteer (himself) with histologically normal gastric mucosa who went on to develop a histologically proven mild illness, acting as a proof of concept of what was later known as *Helicobacter pylori* infection.

This postulate generated serious criticism at that time and Koch was himself aware that his rules were only preliminary. Indeed, he was never able to demonstrate his postulates for cholera because of the lack of a reproducible animal model, despite the fact that he had isolated vibrios as a potential cause of cholera (12). In addition, von Pettenkofer even ingested Koch's cholera vibrios orally in front of his students, without falling ill (12). As summarized recently by Brussow et al., Pettenkofer rejected Koch's hypothesis, instead formulating it as 'X (pathogen) + Y (local milieu) + Z (individual host susceptibility) = disease' (13). Despite countless criticisms frequently linked with the incorporation of epidemiology, Koch's postulates continued to be held through the twentieth century.

Can we go Beyond Culture to Demonstrate Koch's Postulate?

The Place of 16SrRNA Amplification and Sequencing. The advent of 16S rRNA amplification and sequencing gave rise to the hope that molecular tools could replace culture. Whipple's disease is an infectious disease first described in 1907 by George Whipple (15). First considered as a metabolic disease, direct observation by microscopy in 1961 of its form resembling a microorganism and the efficiency of empirical antibiotic treatment led it to be considered as an infectious disease (16). It was only in 1991 that Wilson directly amplified the 16S rRNA gene from a small-bowel biopsy of a patient suffering from Whipple's disease. In 1992, Relman et al. then detected sequences of *T. whipplei* from the tissues of five different patients (17). For Whipple's disease, the establishment of sequences specific to the genome of the bacterium, as a result of its culture, first performed in 2000 (18), made it possible to identify the role of *Tropheryma whipplei* in numerous pathologies, including acute infections (19), outside classic Whipple's disease (20). This work could not have been accomplished based on 16S rDNA alone, given the complexity of the digestive or respiratory microbiota, where this could be demonstrated (19). Quite clearly, moreover, a very high number of false positives were found, initially, in the saliva analyses, due to the lack of specificity of 16S rDNA (21).

Bartonella quintana was first described in 1917 as *Rickettsia quintana*, the agent of trench fever. It was reclassified in 1993 as *B. quintana* following unification of the genera *Rochalimaea* and *Bartonella* (22). The proof of concept of the complementarity of techniques and the need for bacterial cultures, was dramatically brought to light by an issue of the New England Journal of Medicine, published in 1990, in which -(i) the culture of a fastidious bacterium in the blood of a febrile patient later turned out to be *Bartonella henselae* (23) (ii) the observation, using the Warthin-Starry technique, of bacteria during hepatic peliosis (24), also known to be related to *Bartonella henselae*, and (iii) the amplification and sequencing of 16S RNA of a bacillary angiomatosis lesion also revealed *Bartonella henselae* (25). It should be noted that, since then, all the discoveries related to *Bartonella henselae* have been largely due to culture (26,27), which made it possible to find a serology that showed that *Bartonella henselae* was the cause of cat scratch disease. This in turn led to the development of a therapeutic strategy and ultimately to the development of pathophysiological models (22). The replacement of culture by molecular tools to expand the spectrum of microbial pathogens, as currently proposed, was totally irrelevant at that time (2).

The Place of Metagenomics. Metagenomics had promised to render bacterial culture obsolete, by detecting uncultivable microorganisms. The first studies dedicated to the description of the human gut microbiota composition thus revealed that 80% of the sequences were attributed to bacterial species which had yet to be cultured (28). Nevertheless, by comparing different methods to study the same stool samples, including electron microscopy and metagenomics targeting the 16S rRNA gene, Hugon P, et al. demonstrated that metagenomics overlooked a large proportion of Gram negative bacteria (29). Several biases can explain these discrepancies, including extraction bias, primer biases, and depth bias that were subsequently described (30). Indeed, several studies demonstrated that metagenomics could not be used as a diagnostic tool for infectious diseases. First, in an investigation into a Shiga-toxigenic *Escherichia coli* (STEC) O104:H4 outbreak, the use of metagenomics by Loman *et al.* did not detect the causative bacteria in more than 30% of cases (3). As another example, in an investigation of the causative agent of diarrhea using V5-V3 16S RNA amplification, Singh *et al.* did not detect *Campylobacter* and *Salmonella* in 42% and 66%, respectively, of the cases diagnosed by culture, while *Salmonella Shigella* species were never detected