

The greatest steps towards the discovery of *Vibrio cholerae*

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Abstract

In the 19th century, there was extensive research on cholera: the disease was generally attributed to miasmatic causes, but this concept was replaced, between about 1850 and 1910, by the scientifically founded germ theory of disease. In 1883, Robert Koch identified the vibriion for the second time, after Filippo Pacini's discovery in 1854: Koch isolated the comma bacillus in pure culture and explained its mode of transmission, solving an enigma that had lasted for centuries. The aim of this article is to reconstruct the different steps towards the explanation of cholera, paying particular attention to the events occurring in the pivotal year 1854.

Keywords: Filippo Pacini, history of cholera, John Snow, Robert Koch, vibriion

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Introduction

In the 19th century, there was extensive research on cholera: among the topics discussed were microbial vs. miasmatic causes and the relative merits of hygiene, sanitation and quarantine in controlling or preventing cholera's spread, especially among European nations. The most widely accepted explanation of the epidemics was based on the assumption that they were caused by a miasma, which was believed to be a harmful form of 'bad air' or poisonous vapour, filled with particles from decomposed matter. This concept was later replaced by the scientifically founded germ theory of disease, based on the hypothesis that microorganisms can infect the body and provoke specific diseases. The cause of cholera remained an enigma until 1883, when Robert Koch isolated the comma bacillus in pure culture and explained its mode of transmission [1].

History of Cholera

Cholera is an infection of the small intestines caused by various strains of a bacterium known as *Vibrio cholerae*: epidemic cholera is an acute, painful and often lethal disease which

seriously affected almost the whole world during many severe outbreaks in the course of the 19th century [2]. This diarrhoeal disease can lead to death by dehydration of an untreated patient within a few hours, and is extremely contagious in communities without adequate sanitation. Even though it was hard to discriminate cholera from many other diseases associated with diarrhoea and vomiting, the first pandemic of cholera is supposed to have spread out of India in 1817. In 1827, cholera broke out again in India, and in 1829 it appeared in Russia, and then moved towards Europe and the Middle East, finally reaching North America. The third pandemic, which, according to many authors, lasted from 1852 to 1859, involved many Asian, European and American countries [1]. The disease struck again in four other major pandemics [3], spreading through the trade routes and following army troops. Intensive research was performed to understand the virulence properties, giving rise to lengthy debates about the nature and causes of the disease: the scientific world, in fact, was embroiled in debating the merits of the germ theory (disease is caused by a specific organism) and the miasma theory (disease is caused by poisonous vapours in the air). It was observed that the first symptoms might include profuse watery diarrhoea with a 'fishy' smell, accompanied by the vomiting of clear fluids. Then, severe dehydration occurred. Physicians struggled to describe the

disease, discover its cause, and find effective treatments; municipalities published accounts of their own epidemics, and sent representatives to affected regions to determine possible causative factors. Doctors prescribed bleeding, leeching, emetics, and strange therapies, ranging from special vinegars to therapeutic waters, but every treatment was obviously ineffective: many people died, often being buried when they were still alive, as collapse and apparent death were not uncommon in the algid phase [4].

Understanding cholera was a long and difficult process. For the middle of the 19th century, the contribution of many scholars must be taken into consideration; however, the focus of this article will be on three main characters: John Snow, Filippo Pacini, and Robert Koch.

John Snow (1813–1858)

John Snow, a famous anaesthesiologist, is considered to be one of the founding fathers of modern epidemiology, as he theorized that cholera reproduced in the human body and was spread through contaminated water [5].

Snow lived in London, where the sewage system was very poor, and the pervasive stench of animal and human faeces, combined with that of rotting rubbish, made the miasma theory of disease seem very plausible: disease was more prevalent among the lower classes because they stank more, and because the supposed moral corruption of poor people weakened their constitutions and made them more vulnerable to disease.

When the September 1854 cholera broke out in the Soho district, close to Snow's house, Snow mapped the 13 public wells and all of the known cholera deaths around Soho, and noted the spatial clustering of cases around one particular water pump on the southwest corner of the intersection of Broad (now Broadwick) Street and Cambridge (now Lexington) Street. London's water supply system consisted of shallow public wells where people could pump their own water to carry home, and about a dozen water utilities that drew water from the Thames to supply a jumble of water lines to more upscale houses. Snow examined water samples from various wells by using a microscope, and confirmed the presence of 'white, flocculent particles' in the Broad Street samples. Despite strong scepticism from the local authorities, he had the pump handle removed from the Broad Street pump, and the spread of cholera dramatically stopped [6]. Snow subsequently published a map of the epidemic to support his theory, showing the locations of the 13 public wells in the area, and the 578 cholera deaths mapped by home address: the large workhouse north of Broad Street suffered very few cholera deaths, because it had its own well. Likewise, none of the

brewery workers at the brewery one block east of the Broad Street contracted cholera, as they could drink of all the beer they wanted, and the fermentation killed the cholera bacteria. Snow used some proto-GIS methods to support his argument. First, he drew Thiessen polygons around the wells, defining straight-line least-distance service areas for each. A large majority of the cholera deaths fell within the Thiessen polygon surrounding the Broad Street pump, and a large proportion of the remaining deaths were on the Broad Street side of the polygon surrounding the bad-tasting Carnaby Street well [7]. Next, using a pencil and string, Snow redrew the service area polygons to reflect shortest routes along streets to wells: an even larger proportion of the cholera deaths fell within the shortest-travel-distance area around the Broad Street pump [7]. However, despite his attempts, Snow did not identify a pathogen that caused cholera, and everyone continued to believe that cholera was an airborne disease.

Filippo Pacini (1812–1883)

Filippo Pacini was a famous anatomist: his extraordinary expertise in using the microscope enabled him to also pave the way to research in the infectious diseases field [8]. In 1854, when Florence was in the grip of a terrible cholera outbreak [9], Pacini had the opportunity to examine the corpses of the patients who died in the public hospital of Santa Maria Nuova and those of the washerwomen in charge of the cleaning of the hospital linen; he highlighted many similarities in their autopsy records, and, in their faeces and intestinal mucosa, he identified millions of elements, which he called vibrions [10] (Figs 1 and 2). In the Central National Library of Florence, his unpublished memoirs are preserved, where the discovery of the vibriion is reported step by step [11]. By processing his specimens with water, salt, and sublimate, he isolated the agent that he considered to be responsible for the disease, opposing the miasmatic theory [12]. He described the disease as a massive loss of fluids and electrolytes, caused by the local action of the



FIG. 1. *Vibrio cholerae* (recto), by Filippo Pacini, 1854 (University of Florence, Museum of natural History, Biomedical section).



FIG. 2. *Vibrio cholerae* (verso), by Filippo Pacini, 1854 (University of Florence, Museum of Natural History - Biomedical section).

vibron on the intestinal mucosa, and he recommended, in the most severe cases, intravenous injection of sodium chloride diluted in water, a measure that later proved to be very helpful. Pacini introduced a mathematical approach to the study of the disease, describing cholera as a 'quantity disorder' and not a 'quality disorder', as 'all its mechanism is nothing but a matter of losses and reparations' [13]. Pacini's first observation was the lack of a certain 'quantity of villi, and the presence of some superficial corrosions on the same membrane' in the 'choleric intestines', which had the appearance of being 'corroded by moths'. The cause of the disease was, then, the vibron, which 'exists, can be seen, and is not presumed', and is a real element of infection, an 'organic, living substance, of a parasitic nature, communicating, reproducing, and then producing a disease of special character' [14]. However, Pacini, aware that he had no laboratory evidence to demonstrate the relationship between the vibron and cholera, stated with great frankness that he had not even tried to grow it in pure culture, as it would have been necessary 'to attribute the quality of contagion to cholera' [15]. Starting from the clinical observation of the state of apparent death from cholera, Pacini also developed a system for artificial respiration, based on the rhythmic mobilization of the upper limbs in the unconscious patient [16].

Although Pacini had discovered the vibron, his cholera data were ignored by the scientific community and contradicted by influential physicians, who believed in the miasmatic theory, influenced by the localist/contagionist theory of the leading German scientist Max von Pettenkoffer, who considered cholera to be an airborne disease, caused by a combination of three factors: a germ, the local and seasonal conditions, and a constitutional predisposition to infection [17].

Robert Koch (1843–1910)

The work on fermentation by Louis Pasteur (1822–1895) had supported the belief that microorganisms were present in the air and could cause human illness: the pure-culture technique,

based on Pasteur's concept, was then improved by the German scientist Robert Koch, who introduced new techniques and means of cultivating, manipulating and characterizing microorganisms. One of his most important innovations was the use of solid medium instead of liquid to prepare pure cultures of bacteria: liquid medium was easily contaminated by other germs, and colonies of bacteria became mixed up with each other. With solid medium, colonies could be kept isolated. Koch first used ordinary sliced potatoes on which to grow his germs, but later developed techniques involving the use of agar gelatine in Petri dishes [18]. With these techniques of bacterial staining, Koch was able to discover many bacteria responsible for different diseases, such as *Bacillus anthracis* and *Mycobacterium tuberculosis* [19].

In August 1883, Koch travelled with a group of German colleagues from Berlin to Alexandria, Egypt, where cholera was epidemic [19].

Here, a French mission, composed of Isidore Straus, Émile Roux, Edmond Nocard, and Louis Thuillier, was already carrying out the same investigations as the German mission, finding the bacillus that Koch was also to describe, and failing to infect animals. In October, after Thuillier's death, the three surviving members of the mission returned to Paris, where they presented a short report of their findings to the Société de Biologie. In the following year, a definitive account was published, in which they admitted that they could not attribute a specific action to 'the microbe' that they had so often encountered in the greatest number of cholera cases [20].

Koch and his colleagues Georg Gaffky and Bernhard Fischer carried out many post-mortems, finding a bacillus in the intestinal mucosa that was present only in the corpses of persons who died of cholera. He deduced that the bacillus was related to the cholera process, but he was not sure whether it was causal or consequential.

Late in 1883, Koch sailed to Calcutta, India, where the epidemic was still very active [21].

On 7 January 1884, Koch announced that he had successfully isolated the bacillus in pure culture: 1 month later, he added that the bacillus was 'a little bent, like a comma' [22].

He also noted that the bacillus was able to proliferate in moist soiled linen and damp earth, and was susceptible to drying and weak acid solutions. On 2 February 1884, Koch reported from Calcutta to the German Secretary of State for the Interior his reasoned conviction that the vibron found in the intestines and stools of cholera victims was the causal agent of the disease. This was the sixth of seven step-by-step dispatches sent over a period of 24 weeks that provided a description of research in progress, and that were made available to the German press as they were received [23]. It was in his dispatch on 7 January 1884 that Koch admitted that,

despite his success in isolating the bacillus in pure culture, he had failed to reproduce the disease in animals, reasoning correctly that they are not susceptible, but renouncing one of the elements of proof that he had himself fixed in his research, later known as Koch's postulates, which provided a framework for proving the role of microorganisms in disease [24].

The characteristics of the bacillus were listed in Koch's sixth dispatch together with other properties of the bacillus, such as its capability to proliferate in moist soiled linen and damp earth, and its susceptibility to drying and weak acid solutions [22]. Although the bacilli were relatively rare in the evacuations during the early stages of cholera, when evacuations became 'rice water stools', they were present in almost pure culture. In those patients who recovered, the bacilli gradually disappeared from the stools.

Taking Koch's failure to reproduce cholera in animals, Koch's rivals objected that the causal relationship between the comma bacillus and cholera had not been proved: it was only in 1959 that this was demonstrated, when the toxin that caused cholera was discovered [25].

When a conference for the discussion of the cholera question opened in Berlin on 26 July 1884, opinions were divided: was cholera a transmissible disease, or was it 'purely epidemic', affecting large numbers of people simultaneously owing to a conjunction of atmospheric, climatic and soil conditions and of insalubrious wastes [26]?

In Germany, the response to Koch's thesis was mixed, but in France reactions were almost entirely negative, and the most emphatic rejection came from Britain.

At the sixth international sanitary conference, which was held in Rome in May 1885, 28 governments were represented, and Koch was one of the German delegates: the 'theoretical discussion on the aetiology of cholera' was stopped by the British delegation, as Great Britain was concerned about the possibility of hindrance of the intensive maritime traffic through the Suez Canal [27].

However, times were changing, and the British opposition to Koch's views was not unanimous, as it had been previously.

Conclusions

The pivotal year in the history of cholera was 1854: in this year, Snow was able to indicate that cholera was spread through contaminated food or water, but he could not prove his theory. In 1854, Pacini discovered the causative agent of cholera, but his observations remained unknown, and only in 1966 did the International Committee on Nomenclature formally adopt the denomination '1854 Pacini's cholera's vibrio' to indicate the causative agent of cholera [28].

In 30 years, the scientific approach had changed, and the revolutionary work of Pasteur suggested a link between germs and disease, leading the way to Robert Koch to later prove this theory and show how each type of germ caused a specific disease. Koch's discovery had important social consequences. River flooding and swamps offered bacteria a fertile environment: intestinal exposure to contaminated water caused disease in susceptible hosts, and bacteria would return to the water supply through faeces, spreading everywhere through pilgrimages and other types of travel. Koch understood the importance of clean water, and the introduction of filtered water pipes led to a fall in the incidence of the disease. Confirmation of Koch's discovery was provided 8 years later, when cholera ravaged Hamburg, sparing the adjacent town of Altona, where water filtration had protected the town from disease [26]. The importance of water 'purification' and water analysis provided evidence in support of Koch's theory, and showed a way to solve the problem by using the weapons of prevention.

This is why Koch's discovery must be regarded as a public health triumph.

Transparency Declaration

The Authors declare that there is no conflict of interest and that they didn't receive any financial support from anybody.

References

1. Tognotti E. The dawn of medical microbiology: germ hunters and the discovery of the cause of cholera. *J Med Microbiol* 2011; 60: 555–558.
2. Speck RS. Cholera. In: Kiple KF, ed. *The Cambridge world history of human disease*. Cambridge: Cambridge University Press, 1993; 642–649.
3. Pollitzer R. Cholera studies. *Bull Wild Hlth Org* 1957; 16: 295–430.
4. Whitney DH, Branson PF. *The family physician, or every man his own doctor: in three parts*. New York: Sold by N. and J. White, 1835.
5. Snow J. *On the mode of communication of cholera*, 2nd edn. London: Churchill, 1855.
6. Snow J. Further remarks on the mode of communication of cholera; including some comments on the recent reports on cholera by the General Board of Health. *Med Times Gaz* 1855; 11: 31–35, 84–88.
7. Fine P, Victora CG, Rothman KJ et al. John Snow's legacy: epidemiology without borders. *Lancet* 2013; 381: 1302–1311.
8. Bentivoglio M, Pacini P. Filippo Pacini: a determined observer. *Brain Res Bull*, 1995; 38: 161–165.
9. Betti P. *Sul colera asiatico che contristò la Toscana negli anni 1835–36–37–49 comprendente l'invasione colerica del 1855*. Firenze: Murate, 1858.
10. Nardi MG. Discovery of *Vibrio cholerae* by Filippo Pacini, of Pistoia, established in the initial phases of microbiological thought and judged after a century. *Minerva Med* 1954; 45: 1024–1029.
11. Biblioteca Nazionale Centrale Firenze, Carte Pacini, Vol. XVIII, c. 36.
12. Biblioteca Nazionale Centrale Firenze, Carte Pacini, Vol. XVIII, c. 2.
13. Biblioteca Nazionale Centrale Firenze, Carte Pacini, Vol. VI, Fascicolo II, cc. 470–471.

14. Pacini F. Osservazioni microscopiche e deduzioni patologiche sul cholera asiatico. *Gaz Med Ital Toscana* 1854; 6: 397–405.
15. Pacini F. *Della Natura del Colera Asiatico: sua Teoria Matematica e sua Comparazione col Colera Europeo e con Altri Proflussi Intestinali. Memoria del Dott. Filippo Pacini*. Firenze: Tipografia Uccelli e Zolfanelli, 1866.
16. Pacini F. *Sull'ultimo stadio del colera asiatico o stadio di morte apparente dei colerosi e sul modo di farli risorgere. Memoria del dott. Filippo Pacini*. Firenze: N. Martini, 1871.
17. Subba RM, Howard-Jones N. Original observations of Filippo Pacini on *Vibrio cholera*. *Bull Indian Inst Hist Med Hyderabad* 1978; 8: 32–38.
18. Kavica SM, Frehmb EJ, Segalc AS. Case studies in cholera: lessons in medical history and science. *Yale J Biol Med* 1999; 72: 393–408.
19. Howard-Jones N. Robert Koch and the cholera vibrio: a centenary. *BMJ* 1984; 288: 379–381.
20. Straus I, Roux E, Nocard E, Thuillier L. Expose des recherches sur le cholera en Egypte. *CR Soc Biol (Paris)* 1883; 35: 565–570.
21. Yoshifumi T. *Vibrio cholerae* and cholera toxin: from Calcutta to Kolkata. *Sci Cult* 2010; 75: 149–152.
22. Koch R. Fünfter Bericht der Leiters der deutschen wissenschaftlichen Commission zur Erforschung der Cholera. *Dtsche Med Wschr* 1884; 10: 111–112.
23. Koch R. Sechster Bericht der Leiters der deutschen Wissenschaftlichen commission zur Enforschung der cholera. *Dtsche Med Wschr* 1884; 10: 191–192.
24. Mazzarello P. The explicative power of the rules: the case of the Koch's postulates. *Med Secoli* 2004; 16: 293–316.
25. De SN. Enterotoxicity of bacteria-free culture filtrate of *Vibrio cholerae*. *Nature* 1959; 183: 1533–1534.
26. Blevins SM, Bronze MS. Robert Koch and the 'golden age' of bacteriology. *IJID* 2010; 14: 744–751.
27. Howard-Jones N. *The scientific background of the international sanitary conferences 1851–1938*. Geneva: World Health Organization, 1975; 46–57.
28. Feeley JC. Minutes of IAMS subcommittee on taxonomy of vibrios. *IJSEM* 1966; 162: 135–142.