

# ORIGINAL OBSERVATIONS OF FILIPPO PACINI ON VIBRIO CHOLERA

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Robert Koch had been credited with the title "FATHER OF MICROBIOLOGY" as he had first described the tubercule bacilli in 1882, and cholera (comma) bacilli, in 1884, at Calcutta. Subsequently he was awarded Nobel Prize in 1903, for the discovery of the tuberculosis germs. He was also honoured by Kaiser himself, personally awarding the order of the Throne (second class) with star, along with Gaffky and Fischer, who were also awarded the order of the Red Eagle (third class) (1884). The Imperial Health Office presented Koch, with a life size bust of Kaiser, for his epoch making discovery of cholera. Moreover, Parliament voted a monetary award of 1,35,000 Marks to the German Cholera Commission.

A remarkable omission from all Koch's writings on cholera is that he had not mentioned anywhere the earlier pioneer observations of Filippo Pacini (1812-1883) who was working as Professor of Pathology at the Royal Institute of Superior studies in Florence. Pacini had, without any doubt, identified the cholera vibrio as early as 1854 during the cholera epidemic in Tuscany. He incriminated it as the causative agent of the disease and was the first to use the generic name Vibrio, with a legitimate specific epithet "cholera", for the bacterium which is the causal agent of Asiatic cholera. He persistently maintained his contentions for almost three decades, until he died in the very year in which the German Mission set forth for Egypt (1883) in search of the cause of cholera.

In 1854, Pacini published his "Microscopic observations and pathologic deduction on Asiatic cholera" in the Italian Medical Gazette. Pacini described numerous vibrios seen in the intestinal contents of three cholera victims and in a fourth victim, he was struck by their enormous numbers especially in the flocculli consisting of mucus and desquamated epithelial cells. When he teased the masses of cells apart a little, under the microscope, myriads of vibrios emerged. He emphasized in capital letters, that a contagion was an "organic, living substance of a parasitic nature, which can communicate itself, reproduce itself and thereby produce a specific disease". For many years, Pacini fought an unsuccessful battle for the recognition of the vibrio as the cholera pathogen. He remarked bitterly, that his country men would accept his discovery, only when a foreigner had repeated it. He was not so pessimistic as to foresee that the foreigner (R.Koch) who did repeat the discovery would get the sole credit for it.

The discovery of Filippo Pacini was the result of a cholera epidemic which reappeared in 1849 involving a greater part of Europe resulting in more than thousands of deaths a day for several days in succession.

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The severity of this second pandemic in that year gave an impetus to Jean Robert Breant a wealthy industrial chemist to execute a will in which he bequeathed to the Paris Academy of Sciences, the sum of 1,00,000 Francs to be awarded to whomsoever, should find a cure for cholera or discover its cause. The academy thereupon appointed a committee which included Claude Bernard, the celebrated experimental physiologist, and Alfred M. Armand L.P. Velpeau, and an eminent French Surgeon to judge the entries. By 1858, 153 communications, coming not only from France, but from eight other countries, had been received. Pacini submitted his published study of 1854 on "cholera vibrio" and John Snow of his the following year on the epidemiology of the disease "Cholera" (1855). These two contributions, if scientific truth in them were to be recognised, would have been epoch making announcements. Unfortunately these microscopic and epidemiological findings were not given any importance and the two contributions were relegated to oblivion by the Committee of the French Academy as well as by the contemporary scientists.

Arthur Hill Hassall (1854) of Britain also using an identical expression of the findings on the presence of cholera vibrios, reported to the Medical Council of General Board of Health, Great Britain, that he had seen 'myriads of vibriones'. However he did not appreciate the etiological significance of the vibrios, much less the Board of health in Britain.

It is the remarkable character of Pacini, that he persisted in his studies the problem of dehydration and its causative factors in cholera for the next 12 years, in spite of the discouragement he received.

#### **Earlier work on Dehydration and Rehydration :**

Pacini was the first to recognise the proximate cause of cholera resulting in the "loss of 3-4 pounds of water from the blood via the intestines". In 1866, he stated that the most probable mode of transmission of the "molecules" was that "they are propagated by means of drinking water". They passed from the body of one individual into that of another without the individuals having had the slightest contact with each other and remaining at a considerable distance from each other. He pointed out that the muscular cramps and shrivelled appearance of the skin resulted from the loss of intestinal fluid from the tissues. He introduced a mathematical concept of the loss of fluid by quantifying the necessary anatomical facts which form the basis of dehydration. He estimated that the small intestine contained 4 million villi, the total surface area of the alimentary tract was 25,000 sq. cms of which the stomach accounted for 1,000 sq. cms., the small intestine 20,000 sq. cms. and the large intestine 4,000 sq. cms. He established a critical area of 1,000 sq. cms. over and above, which when affected by cholera germs, resulted in diarrhoea.

William Farr in 1867, visited Pacini's Laboratory, in the Hospital of Santa Maria Nuova and was fascinated by Pacini's applications of mathematical techniques to the disease process and described him, as one of the fine microscopists in Italy. Farr included in the 29th annual report of Registrar-General, England, an extremely detailed account

of his mathematical laws on cholera. (1868). In 1871, Pacini expressed himself as "most amply compensated" by such recognition.

In 1876, Pacini again insisted that the pathological changes, in the intestines in cholera could be due only to the "molecular ferment", that infiltrated the mucus membrane, and multiplied there, and was the specific cause of the disease. Referring to the treatment of cholera, he maintained that once the patient had reached the "stage of apparent death" the only therapeutic procedure that remained was the intravenous injections of 1% aqueous solution of sodium chloride.

In 1879, Pacini published in the journal "Losperimentale" full account of his findings and conclusions on the nature of cholera. He brought out in book form a second and amplified version of these ideas in 1880, 3 years before his death.

Pacini not only identified the cholera vibrio but also recognised that it was the pathogenic agent of the disease, that it acted locally on the intestinal mucosa, that its effect was the production of watery dejections that if the rate of transudation from the intestine exceeded the absorption and reabsorption, the blood and soft tissues become progressively depleted of water, that the only therapeutic measure would be intravenous injection of 1% sodium chloride solution, that the symptom mental lucidity even in terminal stages of disease, was remarkable and that there were sub-clinical infections by apparently healthy human carriers. The W.H.O. team while working in Philippines announced the first discovery of cholera carrier in 1962, ignoring the earlier publications of Pacini (Azurin 1967). His brilliant researches and institutions were so far in advance of his time, that they made hardly any impact. His mathematical laws were extraordinarily sophisticated for the time and correspond very closely with today's ideas. All these research papers were published in Italian Medical Journals and his work was not widely known in the other European countries. It seems plausible that R.Koch would not have come across the information on the mathematical laws of cholera enunciated by Pacini eventually published by William Farr in the 29th Report of Registrar General of England (1868).

In the summer of 1883, both the French and German Governments sent medical missions to Egypt to investigate on the spot the cholera epidemic, already past its peak by the time they arrived. The initiative for French mission had come from Louis Pasteur and it consisted of his four disciples. The French workers observed microscopical examination of rice water stools and vomittings of cholera. The German Government deputed Robert Koch to Egypt to study the epidemic. The French workers studied the intestines of the recently dead and made a special mention of a "slender bacillus" about 0.002 mm which invaded the small intestine as far as submucosa without ever penetrating into the blood vessels. In spite of the precaution that Pasteur enjoined upon them before their departure, the brilliant research worker Thuillier succumbed to the

disease cholera. The French commission could not make any meaningful report on the etiological significance of the findings.

The day that Thuillier was taken ill, Koch sent his first report to the German minister stating that a specific sort of bacterium was found in those who had died of the small intestine in cholera victims but not in those who had died from other disease (Koch 1883). Although it was seen in autopsies on only 10 cholera victims the finding was constant. Robert Koch was evidently inclined to consider as the characteristic organism of cholera. He could not demonstrate similar vibrios in other organs of the body such as blood, lungs, pancreas, kidneys etc. Due to the paucity of cases of cholera, he could not complete his work in Egypt and finally he came down to Calcutta on the advice of several English Officials. Koch and his collaborators had carried out investigations on 28 cholera patients and 42 who had died of cholera disease. In his sixth report dated 2nd February, 1884 he described for the first time that the bacilli of cholera is not straight like other bacilli, but slightly curved like a comma. In his seventh and the last report from Calcutta, Koch announced on 4th March 1885 that he had isolated the comma bacillus, from the tank waters in Calcutta, on artificial media and tried experimentally to induce the disease in animals which he carried all the way from Germany (Koch 1884. b).

Koch did not mention Pacini's researches in all his writings. The omission did not pass unnoticed in Italy. Count Vittore Trevisan, a contemporary bacteriologist, protested by publishing an article "On the bacillus of cholera" in 1884. In England the "Lancet" raised its voice in defence of Pacini in an editorial of 2 Aug. 1884. In spite of this impartial recognition by an alien country, it is interesting to note that his name was never even mentioned during the prolonged discussions on cholera at any one of the fourteen International Sanitary Conferences (1851-1938).

The importance of microbial penetration into the intestinal epithelium was experimentally proved by Robert Koch. The necessity of growing the pathogenic cholera germs out side the human intestine was realised by Koch to decide whether the penetration is a primary event followed by denudation of epithelium and consequent diarrhoea or the penetration was a secondary event which followed the denudation due to some other cause. This incisive thinking led to the genesis of a new approach in the field of microbiology i. e. the development of artificial media on which the microbes could be grown. Pacini, however brilliant he was, in explaining the pathogenesis, treatment and microbial penetrations, he did not think in terms of cultivating the germs. The isolation of germs offered an irrefutable evidence for the presence of the causative agent, thus satisfying one of the important postulates made by Koch himself.

Thus, it was incontestable according to Trevisan, (1884) that Koch, primarily a bacteriologist, has the merit of having completed the story by isolating the bacillus in pure culture, but it is equally incontestable that Pacini, primarily a microscopist, has

the merit of having been first to record recognisable description of the organisms to have given the evidence of the importance of its penetration and to have incriminated it as the cause of the disease.

Bianchi (1885) published in a memorial note, after the death of Pacini, the unedited Laboratory notes and illustrations which Pacini made while examining the cadavers of cholera victims in Florence.

Trevisan was convinced of the identity of vibrio cholera of Pacini and Koch's komma bacillus which was recovered from Egyptians with Asiatic cholera. Trevisan was aware that Pacini (1854) had played a bacterium in "zoological genus" *Vibrio Muller 1773*, and he transferred vibrio cholera Pacini to the bacterial genus bacillus as *B. Cholerae*, (Pacini). Trevisan 1884. The generic name Pacini, was proposed by Trevisan (1885), with asiatic cholera organism as the first species named. (The nomenclature is obsolete. A proposal was made by Prof. Hugh to change the generic name to *Vibrio Pacini* in 1964. Loc. cit.).

It is mainly due to the efforts made by Prof. Rudolph Hugh of the United States of America, that Pacini's discovery of the cholera germs received international recognition in 1965, when the judicial commission of the International Commission, on bacteriological nomenclature, decided that the preferred name of this microbe should be "*Vibrio Cholera Pacini 1854*" instead of *Bacillus Virgula* of Koch (1884). Rudolph Hugh (1964) Report of the sub-committee (1972).

### SUMMARY

The original observations made by Pacini (1812-1883) on the vibrio cholera pathogen are examined in the light of the recent historical reviews made by N. H. Jones which were published in *W. H. O. Chronicle*, from April 1974 onwards. Thanks to Professor Rudolph Hugh of U. S. A., Pacini's researches are internationally recognised in 1965 and the microbe is now designated as *Vibrio Cholera Pacini 1854* (*W. H. O. Chronicles* 1974).

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## सारांश

### विब्रियो कालरा पर फिलिप्पो पसिनी के मूल आविष्कार

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पॅसिनी (१८१२—१८८३) द्वारा कालरा जीवाणु पर किये गये मौलिक अध्ययन को एन० एच० जोन्स की ऐतिहासिक समीक्षाओं, जिनका प्रकाशन डब्ल्यू. एच. ओ. क्रानिकल में अप्रैल १९७४ से क्रमशः हुआ, के परिवेश में जांचा गया। पॅसिनी के आविष्कारों को १९६५ में अंतर्राष्ट्रीय मान्यता दी गई जिसके लिए संयुक्त राष्ट्र अमरीका के प्रोफेसर रुडाल्फ ह्यूज बधाई के पात्र हैं। कालरा जीवाणु अब 'विब्रियो कालरा पॅसिनी १८५४' के नाम से जाना जाता है।