Indian Journal of History of Science (2026) 61:133–140
https://doi.org/10.1007/s43539-026-00206-6

ARTICLE

How Sambhu Nath De's discovery changed the fight against cholera

Neelabh Datta[1]

Received: 3 June 2024 / Accepted: 27 January 2026 / Published online: 16 February 2026

Abstract
Cholera is a life-threatening diarrhoeal disease caused by *Vibrio cholerae*, which secretes a toxin that disrupts the electrolyte balance in the intestinal cells. Sambhu Nath De was an Indian medical scientist who discovered the cholera toxin and its mechanism of action using a novel experimental model: the rabbit intestinal loop. His seminal work, conducted in the 1950s and 1960s, challenged the prevailing theories of cholera pathogenesis and suggested the optimal treatment of oral or intravenous rehydration therapy. Although De's research was internationally acclaimed and nominated for the Nobel Prize, he received little recognition in his own country. His death in 1985, left behind a legacy of scientific excellence and humanitarian service. His work on the cholera toxin has opened new avenues for the development of vaccines, drugs, and diagnostics for cholera and other diarrhoeal diseases. He truly is one of the greatest medical scientists of all time.

Keywords *Vibrio cholera* · Toxin · Rabbit intestinal loop · Microbiology · Impact

1 Introduction

The discovery of the cholera toxin by Sambhu Nath De in 1959 was a landmark event in the history of cholera research, as it revealed the molecular mechanism of the disease that had plagued humanity for centuries (Handa et al., 2024). Cholera is a severe diarrhoeal disease caused by the bacterium *Vibrio cholerae*, which was first observed under a microscope by Filippo Pacini, an Italian anatomist, in 1854. In the same year, John Snow, a British physician, showed that the disease was transmitted through contaminated water sources, challenging the prevailing theory that it was caused by 'miasma' or foul-smelling air from sewers (Nair & Takeda, 2011). However, it was not until 1884 that Robert Koch, a German microbiologist, isolated and cultured the comma-shaped bacterium from the intestines of cholera patients in Egypt and India, and named it 'the comma bacillus' (Nair & Takeda, 2011). Koch also proposed three criteria for establishing the causal relationship between a microbe and a disease, known as Koch's postulates. He was able to satisfy the first two postulates, which were to isolate the microbe from diseased hosts and to grow it in pure culture, but he failed to fulfill the third one, which was to reproduce the disease in a healthy animal by inoculating it with the microbe. This was because the 'comma bacillus' did not cause any symptoms in most animals, except for some guinea pigs and rabbits, which developed mild diarrhoea. The reason for this discrepancy was unknown until De solved the mystery in a series of groundbreaking experiments between 1951 and 1959 (Handa et al., 2024). De worked at the Calcutta School of Tropical Medicine, where he dedicated most of his work to understanding the pathogenesis of cholera. He hypothesized that the 'comma bacillus' produced a toxin that affected the intestinal cells, causing them to secrete large amounts of fluid and electrolytes into the lumen, resulting in dehydration and shock. This was tested by preparing bacteria-free filtrates from cultures of *Vibrio cholerae* and injecting them into the ligated ileal loops of rabbits. He observed that the filtrates induced massive fluid accumulation in the loops, mimicking the clinical features of cholera. He also showed that the filtrates contained a heat-labile protein that was responsible for the enterotoxic activity, and that the toxin acted by increasing the permeability of the intestinal mucosa. He named this protein 'cholera enterotoxin' and published his findings in

✉ Neelabh Datta
neelabh24@iisertvm.ac.in

[1] Indian Institute of Science Education and Research Thiruvananthapuram (IISER-TVM), IISER Thiruvananthapuram Campus, Maruthamala P. O, Vithura 695551, Kerala, India

the prestigious journal *Nature* in 1959. By doing so, he not only discovered the cholera toxin, but also provided the first animal model for cholera, thereby fulfilling Koch's third postulate. De's discovery of the cholera toxin was a major breakthrough in the field of cholera research, as it paved the way for the development of effective vaccines, therapies, and diagnostics for the disease (discussed later). His work also inspired many other researchers to explore the molecular basis of other diarrhoeal diseases, such as Escherichia coli and Shigella infections. However, De's contribution to cholera research is often overlooked or forgotten, especially by the international scientific community. The aim of this article is to change that soon, and that De's name will be remembered as one of the greatest cholera researchers of all time.

2 Structural basis of the cholera code

Cholera toxin is a paradigmatic AB_5-type exotoxin whose biological activity, intracellular itinerary, and immunological properties together constitute the molecular core of "cholera toxin". The holotoxin is assembled as a single ~ 27–30 kDa A polypeptide that is proteolytically cleaved into an enzymatically active A_1 fragment and an A_2 linker, plus a homopentameric B subunit (B_5) ring composed of five ~ 11.6 kDa B monomers. The B pentamer mediates the high-affinity multivalent recognition of the ganglioside GM1 receptors, which are densely arrayed on the apical surface of small intestinal enterocytes. The A_1 moiety functions as an ADP-ribosyltransferase that specifically modifies the stimulatory heterotrimeric G protein alpha subunit (Gsα). This constitutively activates adenylate cyclase, thus driving a pathologic rise in intracellular cyclic AMP, and affecting CFTR-mediated chloride secretion with concomitant paracellular and transcellular water efflux. This in turn produces the secretory watery diarrhoea (characteristic of cholera) (De, 1959; Merritt et al., 1994). Structurally, high-resolution crystallographic analyses of the cholera toxin B-pentamer bound to the GM1 pentasaccharide have revealed the molecular determinants of the receptor engagement. A collection of hydrogen bonds and hydrophobic contacts in the GM1-binding pocket of each B monomer and a quinary assembly that creates avidity by presenting multiple binding sites in the pentameric geometry. Mutational and biochemical analysis of the A subunit have mapped the active-site residues required for NAD + -dependent transfer of ADP-ribose onto Gsα, thereby linking atomic structure to enzymatic mechanism and to pathophysiologic outcome (Merritt et al., 1994). Following receptor engagement, cholera toxin is internalized by endocytic pathways and trafficked retrogradely through early and recycling endosomes to the trans-Golgi network and endoplasmic reticulum. There the A_2 peptide and portions of A_1 mediate disassembly of the holotoxin and allow the A_1 fragment to exploit endoplasmic reticulum-associated degradation (ERAD) machinery to translocate into the cytosol in a partially unfolded state.

These events are important because they permit the A_1 catalytic polypeptide to access its cytosolic Gsα target and execute ADP-ribosylation (Sandvig & van Deurs, 2000) Genetically, the canonical structural genes encoding the A and B subunits (ctxA and ctxB) are carried not on the chromosome in isolation but on the filamentous bacteriophage CTX (CT is cholera toxin). Its ability to lysogenize diverse *Vibrio cholerae* backgrounds and disseminate the ctxAB locus, explains how toxigenic potential can spread through horizontal gene transfer. This phenomenon, whose epidemiologic consequences are manifested in the periodic emergence of novel toxigenic clones and in the genetic distinctions between classical and El Tor strains with respect to toxin regulation and phage carriage (Waldor & Mekalanos, 1996). Transcriptional control of ctxAB is achieved via the integrated virulence regulatory cascade machinery centred on membrane transducers such as ToxR (with its accessory partner ToxS) and the cytosolic transcriptional activator ToxT. These together sense environmental and host-relevant signals and coordinate expression of not only cholera toxin but also co-regulated virulence determinants (for example, the toxin-coregulated pilus), thereby coupling phage-encoded gene content to context-dependent expression and pathogenic phenotype (DiRita, 1992). Cholera toxin's immunological footprint i.e., the B subunit's capacity to elicit mucosal anti-GM1 responses and its exploitation as a non-toxic mucosal adjuvant or targeting moiety in vaccine constructs — has been essential in vaccine design strategies ranging from inactivated whole-cell formulations. This are combined with recombinant CTB to live attenuated strains engineered to lack catalytic A_1 activity. These translational applications close the loop from molecular insight to public-health intervention by allowing antigen selection, platform choice, and immunomodulatory strategies that have been deployed in oral cholera vaccines and experimental mucosal vaccines more broadly (DiRita, 1992; Merritt et al., 1994). De's discovery of this toxin in 1959 was a remarkable achievement, considering the difficulties faced by Koch in identifying the causative agent of cholera. Koch, who had successfully isolated and reproduced the tuberculosis pathogen in a guinea pig model in March 1882, within 8 months of his research, could not do the same for cholera, as there was no suitable animal model to confirm the role of the comma bacillus in the disease (Brock, 1988). It was only in the same year as De's discovery, that another research group from Bombay, led by NK Dutta, developed an infant rabbit model for cholera and showed that the disease symptoms were induced by a toxin (Nair & Takeda, 2011). De made crucial discoveries on how cholera causes disease from 1951

to 1959, which changed the knowledge of the disease drastically from that period onwards. The 1953 article of De was a impactful work that started a series of papers that showed the impact of V. cholerae on the intestinal mucous membrane and that resulted in the discovery of cholera toxin (De & Chatterjee, 1953). Before this work, most of the research had consisted of giving the stools of cholera patients or various toxic substances derived from V. cholerae to different animals by various methods and techniques to check for possible systemic or fatal effects, but the results were not consistent. De, however, claimed that the intestinal mucosa was the main site of action of V. cholerae and/or its toxin (De, 1961a, 1961b). Because of the established belief that an endotoxin was the primary toxic factor in cholera, few of the earlier studies had examined the effect of the toxic material on the intestinal mucosa.

3 The formative years of a cholera researcher: De's early life training and research

Coming back to Dr De, Sambhu Nath De was born in 1915 in Garibati, a village on the western bank of the Ganga river, near the former French colony of Chandannagar. He grew up in a large and poor joint family that had lost its fortune due to floods. His father, a devout Vaishnav, ran a small business, while his uncle, the only educated member of the family, mentored him in his studies. After excelling in the Matriculation examination at Garibati High School, De secured a District scholarship, allowing him to attend Hooghly Mohsin College. De excelled in his academics and won several scholarships, including a DPI scholarship during his Inter-Science examination that, which enabled him to pursue medical education at the Calcutta Medical College (Sen & Sarkar, 1990). There, he impressed his teachers, especially Professor M. N. De, a leading authority in Pathology and Bacteriology, who later became his father-in-law by marrying his daughter, Torubala. De graduated with his M.B. degree in 1939 and his Diploma in Tropical Medicine in 1942. He then worked as a Demonstrator of Pathology at the same college, where he collaborated with Professor B.P. Tribedi on several research papers. He also practiced clinical pathology privately to support his parents and siblings. His career took a major turn in 1947, when his father-in-law, who had become the Professor of Medicine at the Calcutta Medical College, arranged for his admission as a PhD student under Professor G R Cameron, FRS (later Sir Roy Cameron), the Head of the Department of Morbid Anatomy, University of London. This was after World War II had ended (Sen & Sarkar, 1990).

The eminent experimental pathologist Roy Cameron, who was associated with the prestigious University College and Hospital in London, came from a humble Australian origin and earned his reputation through hard work and academic brilliance. He did not care for the social status or the privileges that came with his position (Stokes & Drury, 1967). He had a good understanding of India and other Commonwealth nations, and he had a keen eye for talent. He praised De highly, saying that "He is one of the most outstanding of young men I have had through my hands and am prepared to believe that he is probably the best of the experimental pathologists in India... I am confirmed in my belief by other people's opinion" (Sen & Sarkar, 1990). De came to London in 1947 to join the University College Hospital Medical School and to study the changes in the brain caused by experimental hydrocephalus. He faced many challenges in the beginning, as most of the rats that he injected with a fibrin-forming mixture in the 4th ventricle died of respiratory distress, with froth coming out of their mouth and nostrils (Dutta et al., 2015; Sen & Sarkar, 1990). He was very disheartened and depressed by the failure of these experiments in his first three months in London. He wanted to quit and go back to India as soon as possible. But Cameron, who became his mentor, friend, and the main source of inspiration for all his future work, recognised the potential in the accidental finding and advised him to investigate the mechanism of pulmonary oedema (respiratory distress, etc.) that was killing the rats (Sen & Sarkar, 1990). De published his findings on pulmonary oedema (Cameron & De, 1949) and on experimental hydrocephalus (De, 1950). Cameron wrote a letter to M.N. De in early 1948, stating: "S.N. De... passed through a bad period of depression where nothing seemed to go right... he has regained confidence. He is doing excellent work." De achieved remarkable success in London (Stokes & Drury, 1967). He persisted, published, and in 1949, after only two years, he fulfilled the requirements for a research doctorate, obtained a Ph.D. from the University of London, and returned to India. De visited England again in 1955 and 1962. The 1955 visit, supported by the Royal Society, was to present his work on enterotoxigenic Escherichia coli to the Pathological Society. The 1962 visit, supported by the Wellcome Foundation, was to receive his second research doctorate, a D.Sc. degree in Physiology from the University of London. That was the last time De and Cameron met as Cameron died in 1966 (Hall, 2011).

4 From Calcutta to London and back

De, chose to return home from the UK, as he was fascinated by experimental bacteriological pathology, or microbial pathogenesis, and he knew that science must go to the disease. This is particularly notable given that he decided to study cholera pathogenesis, which was unlikely to have happened if he had stayed in the UK. He had already begun

experimental work on cholera before joining Nilratan Sircar (NRS) Medical College, collaborating with one of his former colleagues at Calcutta Medical College, and he reported his initial findings on the action of cholera toxin (De et al., 1951). He had conceived the idea of working on cholera while in England, as he brought with him small appliances for measuring blood pressure of cats, etc. He wrote in the preface to his book on cholera that he had developed a keen interest in experimental bacteriological pathology while working at the University College Hospital Medical School in London, as he observed a fellow worker's study on dysentery toxin (De, 1961a, 1961b). When he became the Chair of Pathology at the Nilratan Sircar Medical College at Calcutta, where the hospital treated most of the cholera cases in and around the city, cholera became his main subject of study. He began his studies on the remarkable pathological changes in the kidneys in cholera cases, which showed renal shunt mechanism in operation – a feature of many other toxic conditions. He published several papers on this topic between 1950 and 1955. Before discussing De's seminal work on cholera, it is worth noting that he published widely on various topics, including rhinosporidiosis, synovial sarcoma, morphoea, hepatic necrosis with haemorrhagic fever, tuberculosis, paratyphi, and tetanus (Waldor & Mekalanos, 1996). Then he published his brilliant work on the discovery of enterotoxigenic *E. coli* in 1956 (De et al., 1956; Sack, 2011). De's interest in cholera research and epidemiology led him to study patient care, epidemiology, infection, physiology, pathology, microbiology, and animal models. He studied the emergence of the El Tor strain biotype, phage resistance, haemolytic variants, haemagglutination, the anomalous Vogues-Proskauer reaction, and strains with unexpected surface features (Sen & Sarkar, 1990).

5 The lost years of cholera research: What happened between 1884 and 1959?

Cholera research remained stagnant until 1959, despite a century of scientific advances in epidemiology, disease causation, and the development of limited intravenous rehydration. The crucial stumbling block for the stagnancy was the erroneous dogma of systemic toxinosis. The failure to identify the cholera toxin for 75 years hindered the progress of basic science and the development of effective vaccines and treatments for cholera, whereas other bacterial pathogens, such as diphtheria and tetanus, were elucidated within five years of their toxin propositions. This delay resulted from various erroneous assumptions and misconceptions about the nature and mechanism of cholera. For instance, De cited Virchow's observation of intestinal epithelium loss in cholera autopsies, and Koch asserted that cholera was caused by a systemic toxin that affected multiple organs and the cardiovascular system, based on the 1st and 2nd Berlin conferences on cholera in 1884 and 1885 (Handa et al., 2024; Van Heyningen, 2019). Van Heyningen later criticized this as "Koch's Blunder" and a "serious matter" on the centenary of the conferences (Van Heyningen, 2019, 1985). Koch also neglected the importance of stool infectivity and the symptoms of diarrhoea and dehydration, which he could not reproduce in animals despite extensive efforts. Koch's influence led the first cholera vaccine developers, Ferrán y Clua in 1885 and Haffkine from 1892, to use only the parenteral route of administration, which compromised the safety, efficacy, and implementation of vaccination (Bornside, 1981; Löwy, 1992). Moreover, the exclusive focus on intravenous fluid replacement restricted the availability of treatment and exposed patients to the risks of unbalanced and non-sterile infusions. Parenteral injections of various cholera preparations in different animals yielded no progress in modelling cholera. By the 1930s, the prevailing view attributed cholera to an endotoxin rather than an exotoxin. In the 1950s, the mucinase hypothesis was popular, and Florey, the Nobel laureate for penicillin, suggested that cholera mucinase removed the mucin layer from the intestinal epithelium, causing a severe internal burn and fluid loss. Van Heyningen and Seal described the field as so confused that they abandoned their review of the literature, which they found utterly useless. This was a major scientific error that left science powerless to prevent the three cholera pandemics that ravaged the world from 1884 to 1964.

6 How De unlocked the mystery of cholera

De demonstrated the existence of cholera exotoxin by using the rabbit ileal loop assay, which he independently rediscovered, along with cholera preparations (Handa et al., 2024). Charles Carpenter Jr. confirmed De's work and concluded that the pathogenesis of cholera was simple: V. cholerae ingested by the host multiplied in the small bowel and produced an exotoxin that stimulated the mucosal cells of the small bowel to secrete large amounts of isotonic fluid, resulting in watery isotonic diarrhoea and rapid fluid loss that caused the clinical manifestations of the disease (Carpenter, 1970). De's discovery, which was urgently needed, did not have an immediate impact; however, after a few years, it was accepted, and cholera became a paradigmatic model for cellular physiology and biochemistry. However, the development of therapy and vaccination was delayed by several misconceptions and conservative attitudes. The discovery of cholera toxin and sodium/glucose co-transport provided the scientific basis for oral rehydration therapy, which was more effective and tolerable than intravenous fluid replacement (Carpenter, 1976). However, it took until 1973 for the World Health Organization to revoke its mandate for parenteral

cholera vaccination, which was unsafe, ineffective, and impractical. It was until 1974, before the first safe, effective, and oral cholera vaccine was licensed after comparative studies (Cash et al., 1974). It is unclear why De's paper was initially ignored. There were only three citations from 1959 to 1963. One citation from 1959 failed to reproduce De's result and proposed a complex toxinosis model involving endotoxin, mucinase, and acetic acid, adding to the confusion and contradiction in the literature (Jenkin & Rowley, 1959). De's work was cited by the researchers who later purified and characterized cholera toxin; however, the lack of recognition and medical conservatism prevented patient care from improving. Carpenter blamed the conservative elements for their irrational adherence to dogma and tradition and accused them of "benign homicide"(Carpenter, 1976). Joshua Lederberg praised De for his humanitarian contributions and his boldness in challenging the established wisdom, and urged that his style of thought should be more aggressively taught by example and precept (Lederberg, 1990). Van Heyningen and Seal called De's, 1959 paper a classic in the history of cholera, cellular physiology, and biochemistry (Hall, 2011).

De's papers on cholera exotoxin, which he discovered using the rabbit ileal loop assay and cholera preparations, were not recognized until years later, when they became citation classics. Garfield coined the term ‘delayed recognition’ and regarded De's, 1959 paper in Nature as a milestone in cholera research. He also suggested that science from developing countries was often ignored in favour of later work from developed countries. However, this may not be the only reason, as researchers often compete for recognition even within the same institution, and developing country scientists may also neglect their peers' work. Another possibility, as quoted by Finkelstein from physicist Max Planck, was that scientific innovations rarely convinced their opponents but rather waited for them to die out and for the new generation to adopt the idea from the start. Thus, when cholera exotoxin became accepted, research advanced rapidly, and De's crucial role was forgotten. De was disappointed and frustrated by the lack of recognition, support, facilities, and staff to further his work on purifying the toxin, obtaining toxin from El Tor strains, and preserving his strain collection. He wrote that it was hard to do and sustain research without adequate personnel and equipment in an undergraduate teaching department in his country (Hall, 2011). This explained why cholera and resource-poor were associated terms. With more institutional investment, De's discovery could have been confirmed and credited earlier, with significant implications. However, the main reason for delayed recognition in this case may be simpler. Van Heyningen and Seal reported that it required the direct involvement of the NIH to assess the cholera exotoxin theory. NIH asked Jack Craig, who had studied the Iota toxin of C. welchii in London, to examine the evidence for cholera toxin in April 1963. Craig's investigations led him to De, and he reported cholera toxin activity at the U.S.-Japan Cholera Conference in Honolulu in October 1965. Dr James A. Shannon, the former NIH Director, realized the importance of the issue for NIH-funded projects on cholera treatment in Dhaka and Kolkata, and he instructed the Cholera Advisory Committee to evaluate the exotoxin theory (Van Heyningen, 2019). Craig praised De's work as creative and novel, and as a catalyst for changing the concepts of secretory diarrhoea pathogenesis (Craig, 1990). De & Chatterje showed that V. cholerae causes fluid secretion by altering the permeability of the intestinal mucosa, using living cultures in the intraperitoneal cavity and the lumen of the ligated intestine of rabbits. They used Evans blue dye, which binds to plasma albumin, to confirm that the fluid leakage was from intestinal capillaries. They also explained why the intraperitoneal fluid had more protein than cholera stools, and why the fluid in the ligated intestine had less protein. Their work led to the discovery of cholera enterotoxin and enterotoxigenic *E. coli*, and the development of a reliable animal model for cholera (Victora et al., 2000). These were key achievements in the history of cholera research. Their work also had a huge impact on public health, as it allowed the invention of oral rehydration therapy, a simple, cheap and effective way to treat cholera dehydration. Oral rehydration therapy reduced the cholera mortality rate from 30% in 1980 to 3.6% in 2000, and saved millions of lives from diarrhoeal diseases, especially among children under 5 years (Victora et al., 2000). Oral rehydration therapy was widely adopted by the World Health Organization and other programmes since 1979. De’s work also opened new fields of study in cellular physiology, biochemistry, and immunology, as it revealed the role of labile toxins from various bacteria in diarrhoea. It also pushed forward the search for immune responses and vaccines against the toxin.

7 How one discovery transformed treatment and vaccination

De’s demonstration in 1959 that *Vibrio cholerae* secretes a diffusible enterotoxin inaugurated a mechanistic reframing of cholera that has had sustained and tangible impact across multiple domains of medicine. It was, however, most conspicuous in clinical management and vaccinology; by establishing that the cardinal pathology of cholera derives from a secreted AB_5 exotoxin. The exotoxin acts at the intestinal epithelium to dysregulate ion transport rather than from an irreversible systemic infection. De’s work provided the essential conceptual substrate for interventions that target the physiological consequences of intoxication (notably oral rehydration therapy) and simultaneously focused molecular

research on the toxin itself as both a pathogenic effector and an exploitable antigenic substrate for vaccine design (Nair & Narain, 2010). At the molecular level, decades of biochemical and structural studies have elaborated the AB_5 architecture. De's discovery pointed toward a single enzymatically active A subunit that catalyses NAD^+-dependent ADP-ribosylation of the stimulatory G-protein alpha subunit (Gsα) and a pentameric B subunit that confers high-affinity, multivalent binding to GM1 ganglioside on enterocyte apical membranes. This integrated structure–function understanding directly informed rational antigen selection and attenuation strategies by identifying cholera toxin B subunit as a non-toxic, highly immunogenic receptor-binding moiety and the A_1 catalytic domain as the locus for targeted inactivation or deletion in live-attenuated constructs (De, 1959). The translational consequences of these mechanistic insights have been concrete: cholera toxin B has been incorporated into licensed inactivated whole-cell oral vaccines as an immunogen and mucosal targeting component (for example the Dukoral formulation) (Holmgren & Czerkinsky, 1993; Merritt et al., 1994). It has also been developed widely as a mucosal carrier/adjuvant in experimental and licensed platforms because of its capacity to elicit protective mucosal antibody responses and to enhance antigen delivery to inductive sites of the mucosal immune system (Holmgren & Czerkinsky, 1993; Merritt et al., 1994). Along with these, knowledge of toxin structure and genetics allowed genetic attenuation approaches. For instance, specific disruption or removal of the A_1 enzymatic domain that underpinned the development and regulatory approval of live oral attenuated vaccines (most notably the single-dose CVD 103-HgR strain marketed as VAXCHORA in some jurisdictions). This thereby translates atomic-level mechanistic knowledge into pragmatically deployable prophylactic products for travelers and outbreak control.[12] Beyond protein-based platforms, the recognition of cholera toxin B's potent mucosal adjuvanticity and carrier properties has stimulated nucleic acid- and vector-based vaccine strategies, in which cholera toxin B sequences or B-fusion constructs are used to enhance mucosal immunogenicity. This showed a conceptual continuity between De's initial observation of an enterotoxin and contemporary efforts to design DNA- and recombinant-vector vaccines that exploit the unique immunobiology of the toxin subunit to elicit protective responses at the intestinal surface (Mosley, 2017; Hou, 2014). The broader public-health impact that links De's mechanistic discovery to lives saved is manifest both in the genesis and universalization of oral rehydration therapy — whose development was made possible by recognizing dehydration as the proximate lethal pathophysiology of toxin-mediated diarrhoea. This is estimated to have averted tens of millions of deaths since its deployment.

8 The other unsung scientists of the battle against cholera

Cholera research has often suffered from the neglect of scientific discoveries, not only in developing countries, but also in the U.S. Van Heyningen and Seal pointed out that in 1883, when Kendrick's cartoon showed the threat of cholera from Europe to New York, the U.S. had no medical science to speak of, while Europe was in the Golden Age of Microbiology (Van Heyningen, 2019). Joseph Kinyoun, who graduated from New York University in 1882 and joined the Marine-Hospital Service in 1886, went to Europe to learn from Elie Metchnikoff and Robert Koch. He came back in 1887 and established a one-room bacteriological "hygenic laboratory" at the Marine-Hospital on Staten Island, NY, modeled after the German facilities (Hall, 2011). He used his European techniques to isolate *Vibrio cholerae* for the first time in America, and confirmed his clinical diagnoses by showing the bacterium to his colleagues with his Zeiss microscope (Armstrong & Kinyoun, 1887). In 1894, he went back to Europe to study antitoxin therapy for diphtheria at Emile Roux's laboratory at l'Institut Pasteur. He earned a Ph.D. from Georgetown University in Washington D.C. and spread diphtheria treatment across the USA. He was then assigned to the quarantine station on Angel Island in San Francisco Bay by the U.S. Surgeon General, where he detected a plague outbreak among the Chinese population of the city. However, the California Governor opposed his control measures, denied the outbreak, and accused Kinyoun. He resigned in 1903 after serving in Detroit, Yokahama, and Hong Kong (Morens, 2009; Winkelstein, 2007). A farewell address praised Kinyoun as a modest and unassuming scientist who advanced medical science and was loyal to his country (Nair & Takeda, 2008). Meanwhile, Congress funded the expansion of the research mission of the Marine-Hospital Service. Joseph J. Kinyoun can be considered the first Director of what later became the National Institutes of Health (Winkelstein, 2007). It is possible that Kinyoun's isolation of V. cholerae and his subsequent prevention of cholera in New York City in 1887 may have initiated the American dedication to biomedical research.

The NIH and the U.S. National Naval Medical Center are located opposite each other in Bethesda. The NIH campus entrance has a coast guard cutter anchor, representing its origin in the Marine-Hospital Service and its role in

[1] European Medicines Agency. (n.d.). *Dukoral (cholera vaccine): EPAR — Product information.* https://www.ema.europa.eu/en/documents/product-information/dukoral-epar-product-information_en.pdf

[2] U.S. Food and Drug Administration. (2016). *VAXCHORA (cholera vaccine, live, oral) — Package insert.* https://www.fda.gov/media/128415/download

preventing the 5th cholera pandemic from reaching New York City. Francis Collins, the former Director, oversaw a budget of more than $30 billion from the James A. Shannon building during his tenure. Shannon was the NIH Director who recognized the importance of exotoxin and supported the Cholera Advisory Committee to test S.N. De's theory (Nair & Takeda, 2008). Joseph Kinyoun, the first American to isolate V. cholerae and a pioneer of the American national biomedical effort, has a lecture series named after him. However, the lasting contributions of Shannon and Kinyoun to the fight against cholera are not widely acknowledged or appreciated.

9 Conclusion

S.N. De was a true pioneer in cholera research who deserves more recognition and appreciation for his contributions to science and public health. He developed a novel method to study the pathogenesis of cholera, using the rabbit ileal loop model, which revealed that the disease was caused by an exotoxin produced by V. cholera (Sen & Sarkar, 1990). He also discovered the existence of enterotoxin-producing Escherichia coli, which are now known as enterotoxigenic *E. coli* (De et al., 1956). His work on cholera toxin opened up new avenues of research in cellular physiology, biochemistry, and immunology. A PubMed search using the keyword "cholera toxin" on November 19, 2009 showed 11,168 publications that were influenced by his work (Nair & Takeda, 2011). He was nominated for the Nobel Prize by Nobel laureate Professor Joshua Lederberg, but his work was largely overlooked in India.

Cholera remains a global challenge, despite almost 150 years of research on V. cholerae since Koch's first isolation of the bacterium and nearly half a century after De's discovery of the toxin. The disease has escalated since the 1990s, as shown by the devastating outbreak in Zimbabwe. Cholera prevention requires good hygiene, sanitation, and access to safe water, but these are often difficult to achieve in low-resource settings. Factors such as population growth, poverty, climate change, and urbanization contribute to the spread of cholera. The disease burden will increase, especially in poor areas where hunger outweighs hygiene. A simple solution would have been elusive without De's practical insight.

Funding I received no financial support for the research, authorship, and/or publication of this article.

Declarations

Conflict of interest I declare that I have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

Armstrong, S.T., & Kinyoun, J.J. (1887). Observations on the cholera bacillus as a means of positive diagnosis. D. Appleton & Company.

Bornside, G. H. (1981). Jaime Ferran and preventive inoculation against cholera. *Bulletin of the History of Medicine, 55*(4), 516–532.

Brock, T. D. (1988). *Robert Koch: A life in medicine and bacteriology* (pp. 96–104). Science Tech Publishers.

Cameron, G. R., & De, S. N. (1949). Experimental pulmonary cedema of nervous origin. *The Journal of Pathology and Bacteriology, 61*, 375–387. https://doi.org/10.1002/path.1700610309

Carpenter, C. C., Jr. (1970). Pathogenesis and pathophysiology of cholera. *Public Health Papers, 40*, 53–56.

Carpenter, C. C. (1976). Treatment of cholera--Tradition and authority versus science, reason, and humanity. *The Johns Hopkins Medical Journal, 139*(4), 153–162.

Cash, R. A., Music, S. I., Libonati, J. P., Craig, J. P., Pierce, N. F., & Hornick, R. B. (1974). Response of man to infection with *Vibrio cholerae*. II. Protection from illness afforded by previous disease and vaccine. *Journal of Infectious Diseases, 130*(4), 325–333. https://doi.org/10.1093/infdis/130.4.325

Craig, J. P. (1990). SN de-an appreciation. *Current Science, 59*(13/14), 637–637.

De, S.N. (1961). Cholera, Its pathology and pathogenesis. Edinburgh, Scotland: Oliver and Boyd.

De, S.N. (1961). Cholera: Its Pathology and Pathogenesis.

De, S. N. (1950). A study of the changes in the brain in experimental internal hydrocephalus. *The Journal of Pathology and Bacteriology, 62*(2), 197–208. https://doi.org/10.1002/path.1700620207

De, S. N. (1959). Enterotoxicity of bacteria-free culture-filtrate of *Vibrio cholerae*. *Nature, 183*, 1533–1534. https://doi.org/10.1038/1831533a0

De, S. N., Bhattacharya, K., & Sarkar, J. K. (1956). A study of the pathogenicity of strains of *Bacterium coli* from acute and chronic enteritis. *The Journal of Pathology and Bacteriology, 71*(1), 201–209. https://doi.org/10.1002/path.1700710126

De, S. N., & Chatterje, D. N. (1953). An experimental study of the mechanism of action of *Vibriod cholerae* on the intestinal mucous membrane. *The Journal of Pathology and Bacteriology, 66*(2), 559–562. https://doi.org/10.1002/path.1700660228se

De, S. N., Sarkar, J. K., & Tribedi, B. P. (1951). An experimental study of the action of cholera toxin. *The Journal of Pathology and Bacteriology, 63*(4), 707–717. https://doi.org/10.1002/path.1700630417

DiRita, V. J. (1992). Coordinate expression of virulence genes by ToxR in *Vibrio cholerae*. *Molecular Microbiology, 6*(4), 451–458. https://doi.org/10.1111/j.1365-2958.1992.tb01489.x

Dutta, Shanta, et al. (2015, Jan.-Mar.) Tribute: Dr. Sambhu Nath De: One of the greatest Indian Scientists. Indian Journal of Pathology and Microbiology, 58(1), 134. Gale One File: Health and Medicine. link.gale.com/apps/doc/A410607018/HRCA?u=googlescholar&sid=sitemap&xid=63199408.

Hall, R. H. (2011). A de in the life of cholera. *The Indian Journal of Medical Research, 133*(2), 146–152.

Handa, A., Choudhari, S. G., & Gaidhane, A. (2024). From pathogen to toxin: The revolutionary work of Dr. Sambhu Nath De in

understanding Cholera. *Cureus, 16*(9), Article e68414. https://doi.org/10.7759/cureus.68414

Holmgren, J., & Czerkinsky, C. (1993). Cholera toxin and cholera B subunit as oral-mucosal adjuvants/antigens: Prospects for vaccine development. Vaccine (review). (See: PubMed entry). https://pubmed.ncbi.nlm.nih.gov/8256498/

Hou, J., et al. (2014). Cholera toxin B subunit enhances immunogenicity of DNA-based vaccines: Evidence and mechanisms. Frontiers in Immunology. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4896579/

Jenkin, C. R., & Rowley, D. (1959). Possible factors in the pathogenesis of cholera. *British Journal of Experimental Pathology, 40*(5), 474–481.

Lederberg, J. (1990). SN de–regicide of reigning dogma. *Current Science, 59*(13/14), 628–629.

Löwy, I. (1992). From guinea pigs to man: The development of Haffkine's anticholera vaccine. *Journal of the History of Medicine and Allied Sciences, 47*(3), 270–309. https://doi.org/10.1093/jhmas/47.3.270

Merritt, E. A., Sarfaty, S., van den Akker, F., L'hoir, C., Martial, J. A., & Hol, W. G. J. (1994). Crystal structure of cholera toxin B-pentamer bound to receptor GM1 pentasaccharide. *Protein Science, 3*(2), 166–175. https://doi.org/10.1002/pro.5560030202

Morens, D.M. (2009). Joseph James Kinyoun: Founding father of American microbiology. 8000 Westpark Dr, Ste 130, Mclean, VA 22101 USA: American Society of Tropical Medicine & Hygiene. In American Journal of Tropical Medicine and Hygiene, 81(5), 31–32.

Mosley, J.F., et al. (2017). Vaxchora: The live attenuated cholera vaccine — Review of safety and efficacy. Clinical Infectious Diseases/Vaccine literature. (See: PMC article). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5614415/

Nair, G. B., & Narain, J. P. (2010). From endotoxin to exotoxin: De's rich legacy to cholera. *Bulletin of the World Health Organization, 88*(3), 237–240. https://doi.org/10.2471/BLT.09.072504

Nair, G. B., & Takeda, Y. (2008). Cholera: The need to translate research knowledge into effective preventive and control measures. *Future Microbiology, 3*(4), 379–381. https://doi.org/10.2217/17460913.3.4.379

Nair, G. B., & Takeda, Y. (2011). Dr Sambhu Nath de: Unsung hero. *The Indian Journal of Medical Research, 133*(2), 127.

Sack, R. B. (2011). The discovery of cholera-like enterotoxins produced by *Escherichia coli* causing secretory diarrhoea in humans. *The Indian Journal of Medical Research, 133*(2), 171–180.

Sandvig, K., & Van Deurs, B. (2000). Entry of ricin and Shiga toxin into cells: Molecular mechanisms and medical perspectives. *EMBO Journal*. https://doi.org/10.1093/emboj/19.22.5943. reviewed concepts of retrograde trafficking and ER translocation relevant to bacterial AB toxins.

Sen, A., & Sarkar, J. K. (1990). Life and work of Sambhu Nath De. *Current Science, 59*(13/14), 630–636.

Stokes, J., & Drury, R. A. (1967). Gordon Roy Cameron. *Journal of Clinical Pathology, 20*(1), 5–6. https://doi.org/10.1136/jcp.20.1.5

Van Heyningen, W. E. (1985). The Berlin cholera conferences of 1884 and 1885. *Trends in Biochemical Sciences, 10*(5), 213–214.

Van Heyningen, W. E. (2019). *Cholera: The American scientific experience, 1947–1980*. Routledge.

Victora, C. G., Bryce, J., Fontaine, O., & Monasch, R. (2000). Reducing deaths from diarrhoea through oral rehydration therapy. *Bulletin of the World Health Organization, 78*(10), 1246–1255.

Waldor, M. K., & Mekalanos, J. J. (1996). Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science, 272*(5270), 1910–1914. https://doi.org/10.1126/science.272.5270.1910

Winkelstein W., Jr (2007). Joseph James Kinyoun: First director of the National Institutes of Health. Epidemiology (Cambridge, Mass.), 18(1), 181. https://doi.org/10.1097/01.ede.0000249523.29233.2a