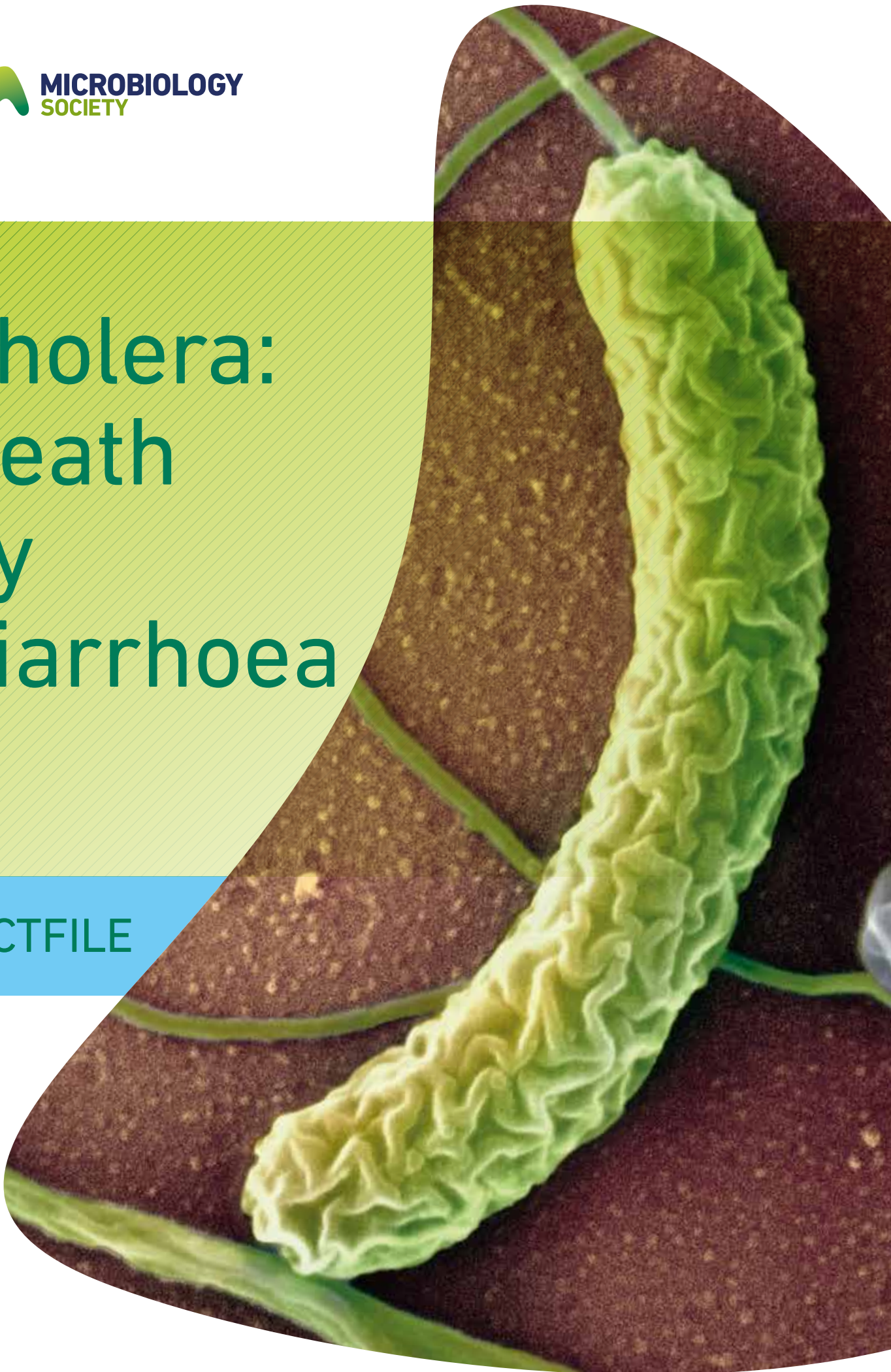


Cholera: Death by diarrhoea

FACTFILE



Cholera: Death by diarrhoea

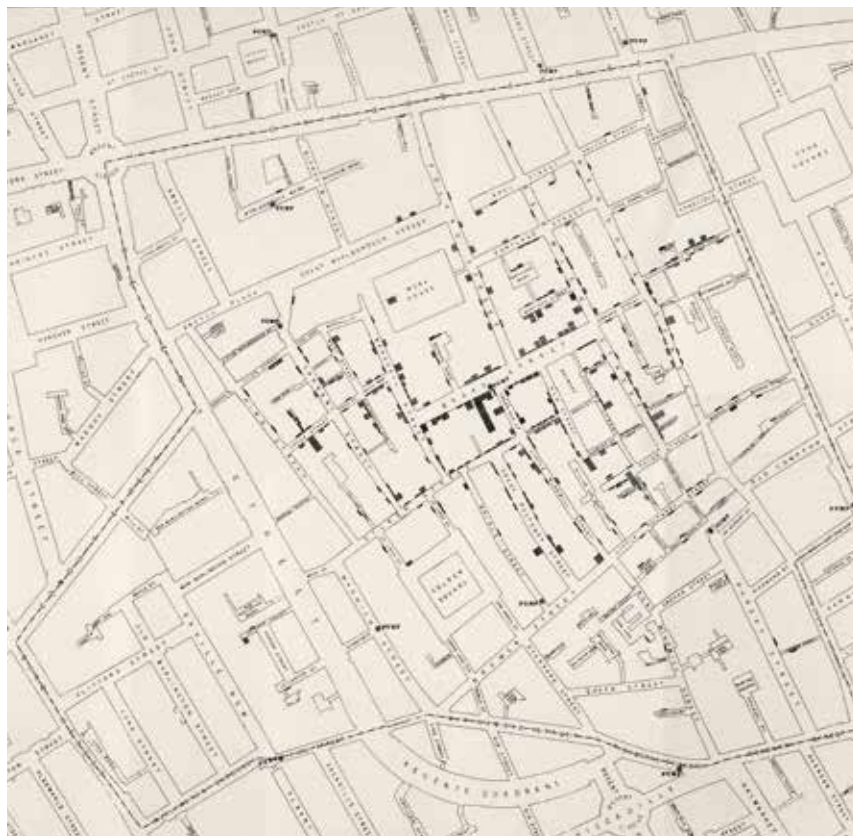
- Cholera is an infectious disease caused by the bacterium *Vibrio cholerae*, which affects the transport of water in the small intestine. The bacterium secretes a toxin, cholera toxin (CT), which causes severe fluid loss from the body into the digestive tract, which leads to dehydration and ultimately death by diarrhoea.
- *V. cholerae* has caused seven worldwide disease outbreaks or pandemics since 1817, killing millions of people and infecting millions more, including four epidemics in the UK accounting for approximately 55,000 deaths.
- Southeast Asia witnessed the first cholera epidemic in 1817, though the condition of 'dehydrating diarrhoea' was mentioned around the time of Hippocrates (460–377 BC). Today, cholera is prevalent in Central and South America, Africa and Asia, though not in the UK as it is usually confined to countries with poor sanitation infrastructure. Worldwide 100,000–300,000 cases of the disease are reported each year with more than 94% of cases in Africa. However, this is likely to be a massive underestimate as many countries in the Indian subcontinent and Southeast Asia do not report cholera incidence; for example, no cholera cases have been reported in Bangladesh, although estimates suggest there may be as many as 1 million cases per year. Realistic worldwide estimates suggest 120,000 annual deaths with 3–5 million people suffering from the disease.



Coloured transmission electron micrograph of a *Vibrio cholerae* bacterium.

Cholera: a history

Prior to 1850 it was thought that cholera was caused by breathing in bad air – ‘*miasma*’ – and that protection was offered by strong smelling substances such as herbs and camphor. John Snow, a prominent London physician was heralded as the ‘Father of Cholera’ when he questioned the miasma theory after treating a number of cholera patients and not succumbing to the condition himself. Snow argued that disease must have entered the body through the mouth via infected food or drink and not via the lungs, after observing that patients became ill with symptoms originating in the gut such as vomiting and stomach ache. He hypothesised that a ‘poison’ was ingested which reproduced in the digestive tract, and in 1849 he published his ideas, at his own expense, in a pamphlet called *On the Modes of Communication of Cholera*. Snow was able to prove his theory in 1854 when he carefully mapped the incidence of cholera during the London 1853–1854 outbreak. Believing that the source was a water pump in Broad Street, Soho he examined water from the pump under the microscope and described the occurrence of “white flocculent particles”. Convinced that these particles were the source of the disease he persuaded the authorities to remove the pump handle; at this time the number of cholera cases locally was already in decline, though it is thought that Snow’s conclusions provided the basis for further preventative measures. John Snow never identified the bacterial



John Snow's cholera map of Soho, London, showing deaths (black lines) due to cholera during the epidemic of 1854.

source of infection; however, through data gathering and statistical analyses he suggested that the water was the reservoir of infection and the mode of transmission. His recommendations of improved hygiene and boiling of drinking water mostly went unheeded by those who did not support his claims as the ‘germ’ responsible for cholera had not yet been observed microscopically.

In the same year that Snow made his observations an Italian anatomist, Filippo Pacini, published a paper entitled *Microscopical observations and pathological deductions on cholera*

describing the causative agent of cholera, and suggesting treatment options. However, the preferred theory in Italy at that time was that of miasma and as such Pacini’s work went unnoticed.

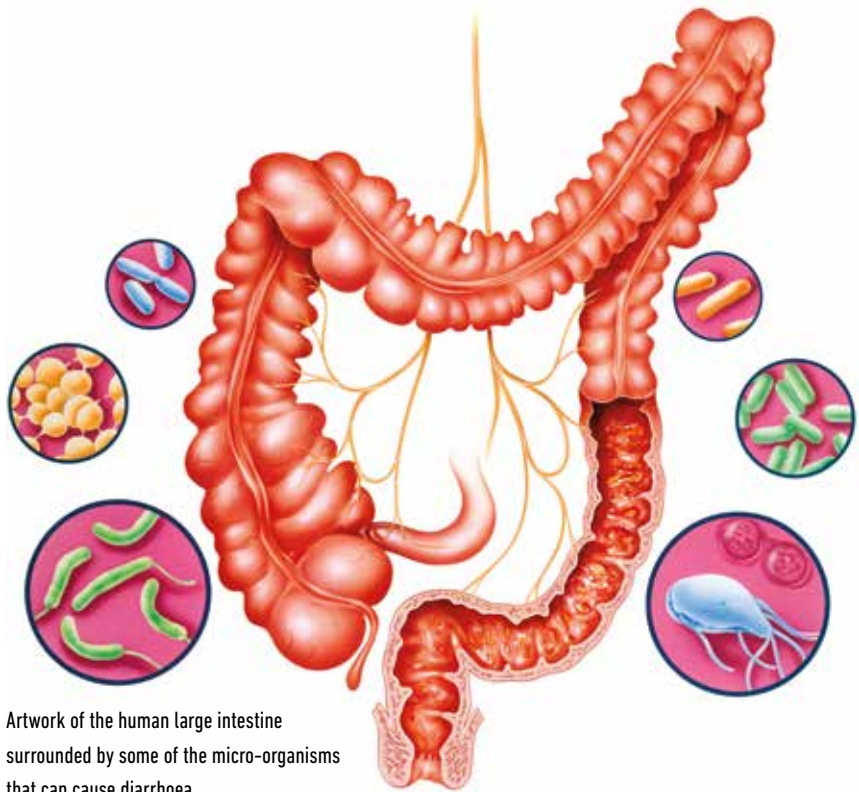
A German bacteriologist, Robert Koch, suggested in 1882 that cholera was caused by a bacterium and that this bacterium secreted a toxin which caused rapid water loss. Koch observed faecal samples and identified a comma-shaped bacillus which he called *Vibrio cholerae* after its vibrating wiggles. Having already made his mark on

bacteriology with his work on anthrax and tuberculosis, and his postulates (see below), which generate a formal proof that a certain micro-organism causes a disease, his ideas and observations on cholera were well received. Pacini was recognised for his work posthumously as the organism was officially named *Vibrio cholerae* Pacini 1854.

What causes cholera?

Organism

Cholera is caused by the curved, rod-shaped bacterium *Vibrio cholerae*. There are more than 100 species or O serotypes of *Vibrio*, only a few of



Artwork of the human large intestine surrounded by some of the micro-organisms that can cause diarrhoea.

Koch's postulates

Robert Koch developed a set of postulates for proving that a specific micro-organism caused a specific disease.



The German bacteriologist Robert Koch, 1843–1910.

Postulate 1:

The suspected pathogenic micro-organism should be present in all cases of the disease and absent from healthy individuals [this postulate is often disregarded as, in the case of cholera for example, it is possible to be a carrier and not show symptoms of the disease (asymptomatic)].

Postulate 2:

The suspected micro-organism should be grown in pure culture (note the correct media must be selected for successful culture).

Postulate 3:

Cells from a pure culture of the suspected micro-organism should cause disease in a healthy animal.

Postulate 4:

The micro-organism should be re-isolated and shown to be the same as the original.

which are pathogenic to humans. They are free-living bacteria, but closely associated with the presence of zooplankton and crustaceans, which are commonly found in brackish waters. *Vibrio* cells are motile, propelled by a single polar flagellum and, as they normally live in neutral or alkaline conditions they are sensitive to acidic pH. The bacteria are tiny; about one five hundredth of a millimetre long which means twenty or more would fit across the diameter of a human hair.

Getting there...

Cholera infection results from the ingestion of contaminated food or water. To survive the journey through the digestive tract the bacterium has to be well adapted.

Passage through the digestive tract

Entry to the body: *V. cholerae* enters the body and makes its way through the digestive system. Approximately two-thirds survive the acidic conditions of the stomach; survivors conserve

'Cholera Dolores'

Asymptomatic cholera infection is unlikely to last more than two weeks; however, long-term carriers have been reported, though they are extremely rare. During the Philippine epidemic of 1962, Dolores, a housewife, suffered a mild attack of cholera while pregnant with her sixth child, and was hospitalised though not treated with antibiotics. It is thought that she contracted cholera from eating contaminated seafood which her husband brought from nearby Bacolod City. Several members of Dolores' family showed symptoms of cholera, though none needed hospitalisation. Dolores intermittently excreted the bacteria so had the potential to cause further infection although this was never observed in her locality. This is possibly due to the fact that she lived in an area where cholera was endemic. Little more is known of this case except that the *V. cholerae* was resident in her biliary tract; it is unknown what factors contributed to Dolores being a carrier for such a long period of time. In 1973, Dolores' carrier state resolved spontaneously.

energy until they enter the small intestine where they begin production of their flagella. The flagellum, which is a long tail-like structure, propels each *Vibrio* cell forward through the mucus layer to the intestinal wall. *V. cholerae* also produces enzymes which digest the layers of mucus, which helps with access to the epithelial cells lining the intestine, as well as detachment from the cells which are being lost due

to the body's defence mechanisms. To conserve energy, on reaching the intestine wall the bacteria stop flagellum production, as propulsion is no longer required, and energy is refocused on the production of hair-like appendages called fimbriae or pili which are formed on the bacterial cell surface. These structures are made of protein and allow the bacteria to attach to the lining of the intestine.

Growth: For symptoms to persist, the bacteria must continue to multiply in the intestine.

Colonisation and Invasion: When they reach the small intestine, the bacteria must 'hold on' and resist the normal transit motions of this region. They do this using their fimbriae or pili. Once attached the bacteria invade the host cell. Production of CT is the final stage in pathogenesis.

Causing trouble...

The period between ingestion and a patient showing symptoms (incubation period) is usually very short and can be as little as 2 hours, although it can take up to 5 days. Most people (75%) infected with *V. cholerae* don't have any symptoms (asymptomatic); however they remain carriers of the disease,

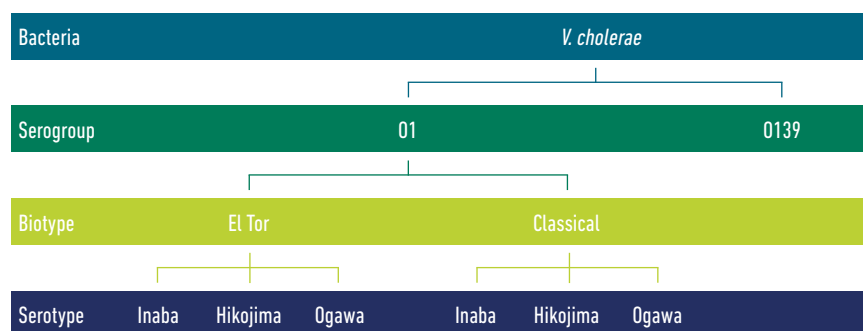
excreting the bacteria in their faeces, usually for two weeks but occasionally for several years as in the case of 'Cholera Dolores'.

Different types of *V. cholerae* infection

Over 100 serogroups (organisms grouped on the basis of their cell surface antigens) of *V. cholerae* exist; however, only two cause epidemic cholera. They are serogroup O1 and O139. Serogroup O1 can be classified further based on phenotype into El Tor and Classical; these are referred to as their biotypes. El Tor is the infective agent responsible for the current pandemic; Classical has not been identified since the mid-1990s. *V. cholerae* O1 biotypes can be further classified into serotypes based on the results of a serum agglutination test; there are three O1 serotypes, Inaba, Ogawa and Hikojima. *V. cholerae* O139, a relatively new serogroup, has not reached epidemic potential so far, though its occurrence is being closely monitored as its pattern of infection and capacity to survive in water suggest that it may be both more infectious and virulent than *V. cholerae* O1.

It is worth noting that the gene for CT is carried on a bacteriophage (a virus which infects bacteria) inside *V. cholerae*.

Classification of *V. cholerae* infection





Bacteriologist holding a Petri dish containing colonies of *Vibrio cholerae* bacteria.

So, in theory if this virus were more mobile it might be possible for a new serotype to emerge again in the future. Alternatively, *V. cholerae* species could swap genes involved in the production of O antigens as has happened for O139 and O37. These serotypes have a genetic fingerprint resembling O1 serotype strains, but in both cases the O1 antigen gene cluster has been replaced by a non-O1 gene cluster.

Symptoms

Most people with cholera are asymptomatic and, of those that do develop symptoms, 80% have mild diarrhoea which is difficult to distinguish from that caused by other

pathogens, such as viruses. However, all of these people carry *V. cholerae*, excrete it and can spread it to others. Severe cholera (cholera gravis), which accounts for fewer than 10% of cases, is characterised by:

- rapid onset of violent watery diarrhoea – the diarrhoea is 'straw coloured' with flecks of mucus and is often described as resembling rice water
- vomiting
- extensive dehydration
- leg cramps

As fluid is lost the blood thickens and the skin becomes blue-grey in colour; patients also begin to suffer lethargy,

a lack of consciousness, confusion and occasionally fever. It is this rapid dehydration – as much as 1 litre of fluid can be lost every hour – which can prove fatal within 24 hours of developing the disease. In children, the rapid loss of fluid can lead to severe salt imbalance, which can cause convulsions and cardiac arrest in addition to the symptoms described. The fatality rate in untreated cases can be as high as 30–50%. If patients are given rehydration therapy, then the death rate is below 1%.

Laboratory methods for diagnosis of *V. cholerae*

Many diseases cause diarrhoea; however, the production of frequent watery stools requires faster treatment due to the high risk of dehydration. Looking at a stool sample under the microscope can help the medical practitioner make a diagnosis. However, this is of little use to patients as those with any diarrhoeal disease receive the same treatment, i.e. fluid and electrolyte (salts and sugars) replacement.

Conventional culture methods are the 'gold standard' for diagnosis of *V. cholerae*. Stool samples or rectal swabs on TCBS (thiosulphate citrate bile salts) agar (*V. cholerae* colonies appear yellow as they ferment glucose). Suspected colonies are selected for further analysis with biochemical and serological tests such as slide agglutination.

In cholera endemic areas, where laboratory facilities are often limited, a rapid immunochromatographic dipstick test can be used to diagnose cholera. It is very simple to use; the dipstick is dipped into a stool sample. If two red



Two girls washing dishes in muddy river water on the Niger River in Mali. Using unclean water can lead to infections from parasites, bacteria or other microbes.

lines appear on the dipstick, then the patient has cholera, if only one red line appears, the test is negative. It takes between 2 and 15 minutes for the test to develop.

Retrospective diagnosis can be performed by analysis of the blood for antibodies against *V. cholerae* and CT. Although identification of *V. cholerae*

is not often used in treatment, this information helps to prevent further outbreaks.

Transmission of cholera

Cholera infection requires a large dose of bacteria and is transmitted by the faecal-oral route, for example by drinking from a water supply

contaminated with infected excrement. The bacteria can also be spread to food if infected people don't wash their hands thoroughly after going to the toilet and before food preparation. Infection is often noted after a funeral of a cholera victim where contamination occurs as a result of poor hygiene in food preparation.



Drinking water supplies being distributed during a cholera epidemic in 19th century Hamburg, Germany.



Patients being treated in a cholera treatment centre, run by Médecins Sans Frontières, in Port-au-Prince, Haiti.



Hartmann's solution is used to replenish body fluid and restore normal salt concentrations for patients who have become severely dehydrated after an illness.

The environment can also be a reservoir of infection as the bacteria are present in brackish waters, often in association with aquatic organisms at a low level, and sometimes in an unculturable state. Shellfish living in contaminated water can transmit cholera too. They are filter feeders and, as they strain the water for food, the bacteria become concentrated inside them. Anyone consuming shellfish that are not properly cooked can become ill.

Treatment

Treatment of the symptoms of cholera focuses on fluid replacement as water alone is not easily absorbed by the body. The World Health Organization (WHO) recommends clean water and rehydration salts to restore electrolyte balance. Oral rehydration therapy (ORT) employs rehydration salts, which are readily available in pharmacies; these are often in short supply in areas of high infection rate. ORT has been revolutionary in cholera treatment and, if properly used, it hugely reduces both the need for hospitalisation and mortality. Some cases may be treated with antibiotics although generally the individual's immune system is left to destroy the pathogen. In extreme cases, Hartmann's solution (a solution of sodium, chloride, lactate, potassium and calcium ions, which is isotonic with blood) is administered by intravenous injection to replace body fluid and mineral salts.

Prevention

Good sanitation, clean drinking water and improved hygiene practices, such as washing hands after visiting the

Food	Drink
Cook all foods thoroughly	Use bottled, boiled, or chemically treated water to wash dishes, clean teeth and prepare food
Raw fruit and vegetables should be avoided unless you thoroughly wash and peel them yourself	Only drink bottled water that has the seal still intact
Ice cream from doubtful sources may also be contaminated therefore should be avoided	Avoid ice cubes in drinks
Food must be properly prepared and still hot when it is served	Boil unpasteurised milk before you drink it
Be careful when eating food from street stalls	
Avoid salads as they may have been washed in contaminated water	
Avoid raw fish and shellfish	



Clean and dirty water in glasses.

toilet and before preparing food, are all strategies that need to be followed to prevent the outbreak of cholera. The table above shows some tips for travellers on how to avoid infection, from food and drink, when visiting an area where cholera is present. A simple rule is: *boil it, cook it, wash and peel it or forget it.*

Physiological differences affecting infection

Cholera infection can be spectral in that symptoms can vary with dose, serogroup, biotype, and serotype, though physiological variation between people can also play a part. It is thought that those who have reduced or non-existent stomach acid due to disease or ailment are more susceptible to cholera. In a healthy individual, the stomach acid

acts as a first-line of defence against infection and kills many *V. cholerae* cells before they travel to the intestine. Another possibility for physiological variation among infected individuals may be the availability of surface receptors for CT on the host cell surface, though this has not been proven.

Vaccines and Immunity

Oral vaccines show long-lasting protection against cholera with few side-effects, although they provide insufficient protection for children under the age of 2. Vaccines, however, are only advised for preventative use rather than as a method for controlling outbreaks and should be used in tandem with standard prevention and control measures. Also immunity, natural or artificial, is serogroup-specific; for

example immunity provided against *V. cholerae* O1 does not protect from O139. The WHO recommends vaccination for people occupying slums or refugee camps due to high density populations and increased risk of disease spread.

In 1894, soon after Koch's identification of cholera, an injectable, whole-cell, killed vaccine was developed that induced 48% protection for three months against *V. cholerae* serotypes Inaba and Ogawa. The vaccine, initially trialled in India, was never endorsed by WHO, and is no longer available.

Protective immunity in those exposed to cholera is induced almost exclusively by antibodies produced in the intestine. These antibodies prevent bacterial colonisation and multiplication, and they inactivate the CT. Immunoglobulins IgA, IgG and IgM have

all been detected, although IgA are the most important. The antibodies prevent the CT from binding with the receptors on the cell surface. Natural immunity is provided by IgM followed by a switch to IgG. There is a 3-year period post infection where patients remain immune from cholera as a result of acquired, natural immunity.

In areas where cholera is endemic such as Bangladesh, infection rates are low among adults when compared to the children in the same area, whereas in areas where new epidemics arise rates are higher in the adult population. This discrepancy illustrates a resistance linked to the presence of circulating vibriocidal antibodies to *V. cholerae*.

Future

Current research into cholera is focused on understanding the CT itself. Another area of anti-diarrhoeal research is development of a pill that will prevent the diarrhoea post-infection. Two drugs, chlorpromazine and nicotinic acid, have been shown to be effective in animal models; though

Effective oral vaccines available against cholera

WC/rBS (Dukoral)

This is an oral killed, whole-cell *V. cholerae* O1 vaccine with the recombinant B (binding) subunit of CT. Trials have shown high levels of protection (85–90%) over a period of 6 months for 2 doses of the vaccine in all age groups, though protection declines after 6 months in young children and remains at about 60% in older children and adults after 2 years. Protection is provided against *V. cholerae* O1 serotypes Inaba and Ogawa, and biotypes Classical and El Tor. Dukoral also provides short-term protection against *Escherichia coli* enterotoxin, which is of added benefit to travellers. This vaccine is licenced in Europe.

Bivalent variants of WC (Euvichol, m-ORCVax and Sanhancol)

These are cheaper versions of the oral killed Cholera vaccine WC/rBS and are licenced in the Republic of Korea, Vietnam and India respectively. These vaccines lack the B subunit of CT and contain twice as many killed *V. cholerae* cells, including cells of serotype O139. Two years after vaccination, the efficacy is approximately 66% in all age groups including infants. The WHO has built a stockpile of oral killed Cholera vaccines

to assist with the containment of cholera outbreaks and epidemics in susceptible populations. The stockpile has already been used to great effect.

CVD 103-HgR (Vaxchora™, previously Orochol)

This vaccine consists of an attenuated, live, genetically modified *V. cholerae* O1 Inaba strain that has been engineered to produce part of the CT. It confers high protection (95%) against O1 (Classical and El Tor) in virgin volunteers, i.e. those not previously exposed to the bacterium. Protection in endemic regions has not

been shown in an effective trial, though retrospective studies have shown protection in an ongoing outbreak in Micronesia, a group of islands in the Western Pacific. Orochol was the only vaccine available as a single dose, which, due to administration logistics, is more viable for pre-emptive and long-term outbreak control in ongoing field conditions according to WHO. However, due to a lack of evidence for its effectiveness, the vaccine was withdrawn in 2004, now redeveloped as Vaxchora™, which is in the advanced stages of application for a US licence.

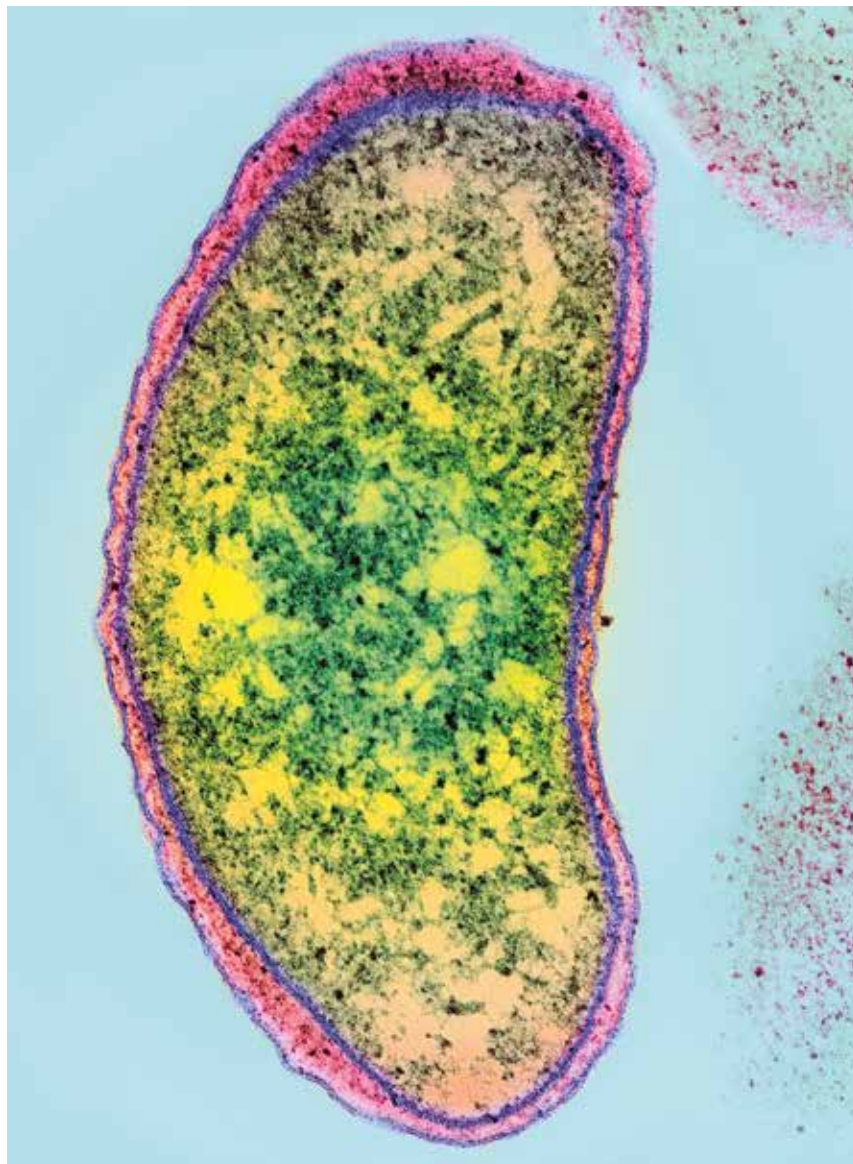


Child being given an oral vaccine in Afghanistan.

the mechanism is yet to be understood. Research into development of a live attenuated Cholera vaccine continues, and it is currently in clinical trials in Australia and the US. Following promising field trials of the bivalent oral killed Cholera vaccine in Bangladesh, the introduction of this vaccine in the national vaccination programme is being considered.

Education

In order to control and ultimately stop the spread of a cholera outbreak, community education is paramount. Many of the basic hygiene messages, while perhaps simple to relate, are often difficult to implement due to cost. Therefore, alternative solutions are required to limit transmission, for example the WHO suggests the addition of lime juice to food and water to inactivate *V. cholerae*. Ideally in communities most affected by cholera, awareness campaigns continue throughout the year, increasing in frequency as the cholera season approaches. Clear information is presented with messages adapted for culture, social and economic circumstances, and the information is delivered graphically, by radio broadcast or as talks in areas where people are waiting or congregated.



Coloured transmission electron micrograph of a *Vibrio cholerae* bacterium.

Sources of further information

Centers for Disease Control www.cdc.gov/nczved/divisions/dfbmd/diseases/cholera

European Centre for Disease Prevention and Control www.ecdc.europa.eu/en/healthtopics/cholera

Net Doctor www.netdoctor.co.uk/travel/diseases/cholera.htm

BBC www.bbc.co.uk/scotland/education/int/geog/health/health/cholera/causes

World Health Organization www.who.int/cholera/vaccines/double/en

Textbook of Bacteriology www.textbookofbacteriology.net/cholera.html

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Front cover and right Coloured scanning electron micrograph of a *Vibrio cholerae* bacterium.

