The environmental transmission of cholera

by Ann Storey

Cholera transmission has been linked to water for over a hundred years, but faecal contamination is not the only route to disease; new research points to other danger areas.

SINCE THE WORK done in London in 1854 by John Snow, and later by Robert Koch, it has been known both that water is an important factor in the transmission of cholera, and that water from public supplies had been implicated in earlier pandemics (see Table 1). While water from treated public supplies does not appear to be a risk factor in disease transmission in the present seventh pandemic, untreated surface water from lakes, ponds, canals, and even wells continues to be an important source of infection.

Table 1. Cholera pandemics up to the present day.

Pandemic number	*Dates*
1	1817-23
2	1829-51
3	1852-59
4	1863-79
5	1881-96
6	1899-1923
7	1961-

It had generally been thought that water sources were contaminated by infected human waste, and that the general spread of an outbreak was via the faecal-oral route, as is the case with many other important infections, such as typhoid, hepatitis A, and most other diarrhoeal diseases. The evidence for this was a general failure to detect the cholera organism in water, except when cholera cases were in the immediate vicinity. While there is no doubt that the faecal-oral route is of major importance in the development of secondary cases and in the spread of the disease in most areas, it does not explain outbreaks which occur where this route is unlikely, such as in Louisiana in the United States. Using improved laboratory techniques, *Vibrio cholerae* has now been isolated in water even when other faecal bacteria, in particular *Escherichia coli* — the most widely accepted indicator of pollution by faeces — were not present. This indicates either than *V. cholerae* can survive longer in the environment than other faecal organisms, or that it can exist as an environmental organism in its own right.

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An adaptable organism

Until the late 1970s, the normal habitat of *V. cholerae* was thought by most to be the human intestine, and it was not thought to be capable of surviving for more than a few days outside this habitat. Other vibrios, both pathogenic (disease causing) and non-pathogenic, normally inhabit estuarine and marine environments, and so do some types of *V. cholerae*.

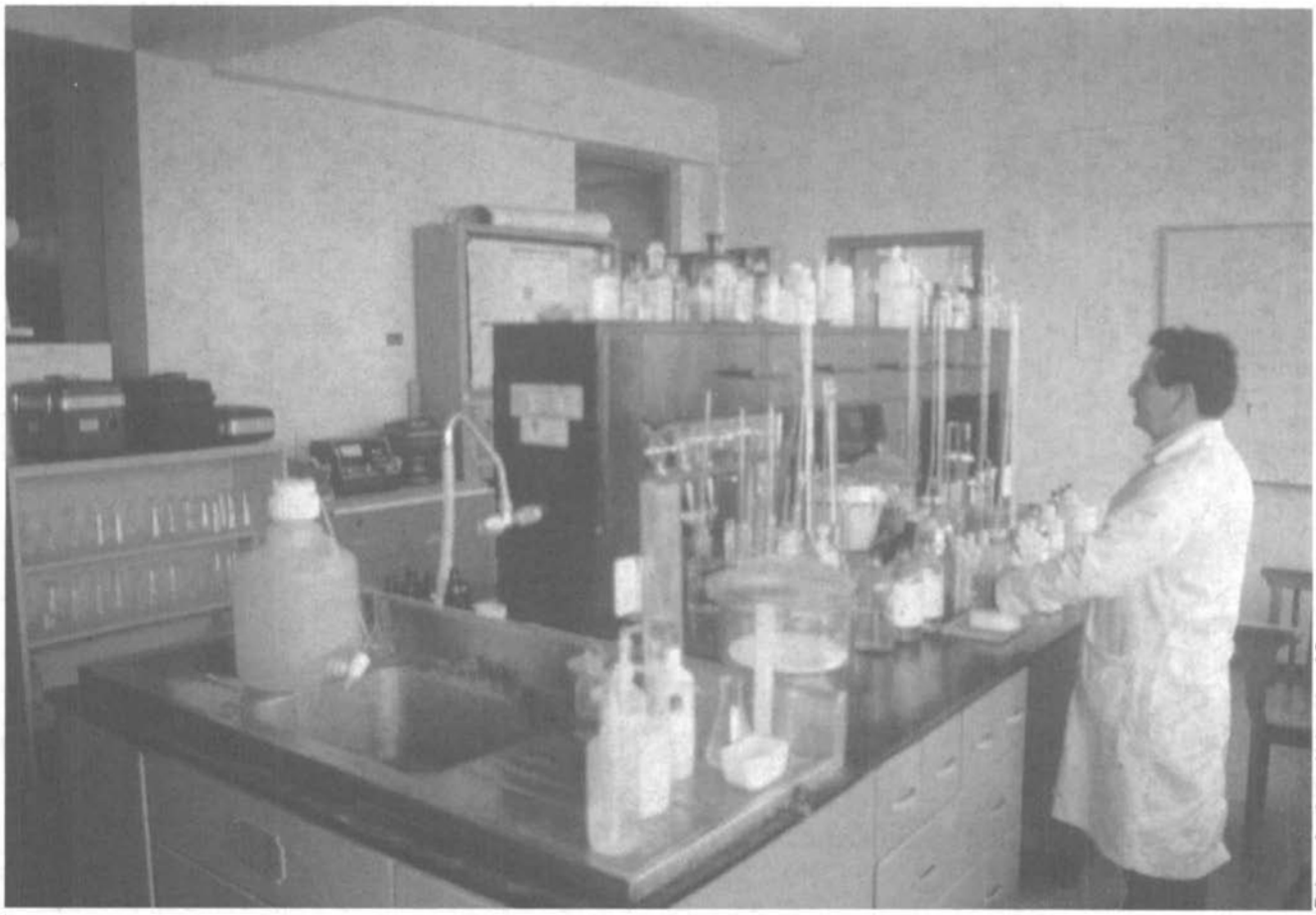
The disease cholera is caused by the release of a toxin known as CT (choleragenic toxin) which not all *V. cholerae* can produce. *V. cholerae* from any subgroup may or may not be able to produce toxin but, unfortunately, testing for either the production or presence of the toxin requires specialist techniques and equipment. Finally, some *V. cholerae* can produce toxins other than CT and may cause other, milder, illnesses.

Table 2. Characterization of *Vibrio cholerae*.

Serotype	*Includes*
V. cholerae 01	El Tor and Classical biotypes; Inaba, Ogawa, and Hikojima serotypes. Both cholera toxin positive and negative strains.
V. cholerae non-01	013 serotypes including 0139. Other non-agglutinating *V. cholerae*. Both cholera toxin positive and negative strains.

The species *V. cholerae* is made up of many types or subgroups. The subgroup responsible for clinical cholera is known as *V. cholerae* 01, which includes two biotypes: 'Classical' and 'El Tor'. Both of these can also be broken down into three serotypes, Ogawa, Inaba, and Hikojima (see Table 2). It is easy in a laboratory to test whether a *V. cholerae* is 01 or not: those found positive are designated 01s, those showing negative are designated *V. cholerae* non-01 (formerly 'non-agglutinating *vibrios*', although they may agglutinate other antisera if these are available). Most *V. cholerae* isolated from the environment are non-01s. These were not usually thought to cause cholera, although some were found to produce all the known toxins, including choleragenic toxin, and had caused small numbers of cases of diarrhoeal and cholera-like diseases.

In 1992, a major outbreak of cholera caused by *V. cholerae* 0139, a non-01 type, occurred in India, followed by another outbreak of the same strain in 1993 in Bangladesh, sparking fears of an eighth pandemic before the seventh was even over. At least 150 000 people



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The existing methods for quantifying cholera are slow and complex.

are believed to have been affected by the end of 1993.

While non-01 *vibrios* may be isolated in quite large numbers from estuarine and marine waters, 01s are usually present only in low numbers, and most of these appear not to be producing toxin. Much research has been done on the survival of *V. cholerae* in different water types, including variations in salinity, organics, and temperatures. This research has found that when *V. cholerae* is added to water, it adapts to its new environment by becoming smaller and slowing its metabolism to take into account its new nutrient-poor surroundings. It also becomes harder to grow and detect by conventional laboratory methods. Surveys have indicated that numbers of *V. cholerae* are at their highest when water temperatures are between 20°C and 35°C, and at salinities between 1.0 and 2.5 per cent, although this is very variable. Levels of organics above 1.0 milligram per litre, which can often be found in runoff from agricultural and urban areas, decreases the organism's need for salt. In addition *V. cholerae* is often found in higher numbers in aquatic plant life and in some fauna, especially crustacea and filter feeders such as oysters.

When estuaries known to be contaminated with *V. cholerae* are sampled over a period of a year, the organism is shown to be seasonal in its appearance, generally occurring during the warmer months of the year or following the monsoon, depending on the part of the world. While in contact with low salinity and relatively warm water the organisms remain culturable. Once in contact with full-strength cold seawater (<10°C) they rapidly become non-culturable, but can still produce toxin in laboratory tests. In fact toxin-producing strains do not lose the ability to produce toxin after their introduction to an aquatic environment.

Cholera in freshwater

Surface water that is generally used for drinking has a salinity of less than 0.1 per cent and organics below the levels

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Strict hygiene rules will prevent the contamination of water sources.

Cholera has a high incidence in fishing communities in Bangladesh, and many secondary cases arise there.

which would negate the cholera organisms' salt requirement. This would seem to rule out colonization by *V. cholerae*, but *V. cholerae* 01 can be isolated from freshwater sources and, as in estuaries, it appears to survive there by adhering to water plants, sediment, and crustacea. It has been found in algae, water hyacinths, and in many other aquatic plants. In crustacea such as crabs and shrimp, *V. cholerae* attaches to the chitin in their shells. It is not surprising therefore that another of the major ways of contracting cholera is by eating shellfish. This is the main way that cholera is contracted in the southern states of the USA, where *V. cholerae* 01 is known to be present in the estuaries.

V. cholerae does not tolerate dry conditions, and while it will survive in wet soil, it rapidly dies when it dries out. Likewise, it will survive in wet human waste, but disappears when that drys. It is also killed off during some sewage treatment processes, especially anaerobic digestion.

Transmission by higher animals does not appear to be important, although *V. cholerae* non-01 has been isolated from some water fowl and fish. It may be that waterfowl play a role in the transmission.

As previously discussed, *V. cholerae* can maintain itself at low levels in the aquatic environment, normally not higher than 50 organisms per litre. The infecting dose for humans, however, is between 10^2 and 10^8, depending on the individual. So for the organisms in the water to cause

disease, they must first multiply somewhere. This could happen on food or in stored water, or on infected shellfish. *Vibrios* can stick to the shells of crabs in very high numbers, as well as colonizing the crab's hind gut, which also forms part of its shell. This could then lead to a few primary cases caused by people eating infected crabs. In countries with good hygiene and support services, such as in the USA, the number of secondary cases (caused by people infected by the person infected by the crabs) would be low, but in countries where services were poor or non-existent, then the number of secondary cases resulting from the faecal-oral route could be very high. Cholera rates remain high in endemic areas even when the population gets its drinking-water from supposedly uncontaminated tubewells, because often the people are still bathing in contaminated surface water and eating shellfish from contaminated estuaries. In Bangladesh, where cholera is endemic and where it may have originated, the peak cholera seasons in rural areas are between March and April, and after the summer monsoon. This is not the season of peak salinity (which is pre-monsoon), but it is associated with a sudden growth in algae and animal plankton, and a rise in both water recreation and the consumption of shellfish. It has a high incidence in fishing communities and many secondary cases originate there.

Testing

V. cholerae can be isolated from the environment using modifications of the standard techniques used to culture faecal bacteria from water. These methods are described in reference books and involve concentrating a given volume of water before testing for the organism.[4] The most suitable concentration method is filtration using membranes, borosilicate glass, or diatomaceous earth. A filtration technique using plastic containers packed with gauze has been used successfully by some workers. After concentration, the filter material is added to a given volume of an enrichment medium, usually alkaline peptone water. From here the organisms can either be cultured directly or a statistical Most Probable Number technique can be performed. Alternatively, *V. cholerae* can be isolated directly from the membranes by laying the filtration membranes straight onto the selective media. (This method grows lower numbers of organisms than the other methods.) Work being carried out at the Robens Institute has shown that if the membranes are initially cultured onto an alkaline peptone water-soaked pad for four hours before transferring the membrane onto selective agar, then the number of organisms isolated is comparable to that obtained by the more laborious standard methods. This method could be useful for workers in isolated areas because it does not require a full laboratory set-up, but can make use of a field-test kit.

None of these methods will confirm *V. cholerae*'s subgroups. For this it is necessary to carry out more specialized tests in a laboratory. These include confirmation that the organism is indeed *V. cholerae*, subgroup determination, and the detection of toxin-producing strains.

Other techniques are necessary to discover non-culturable organisms; both fluorescent antibody tests and DNA probes have been used. These techniques are both specialized and expensive and are outside the scope of most workers in the field. They do provide much higher counts than those obtained by culture alone, however, and so are of value in researching further the environmental habitat of *V. cholerae*. ●

References

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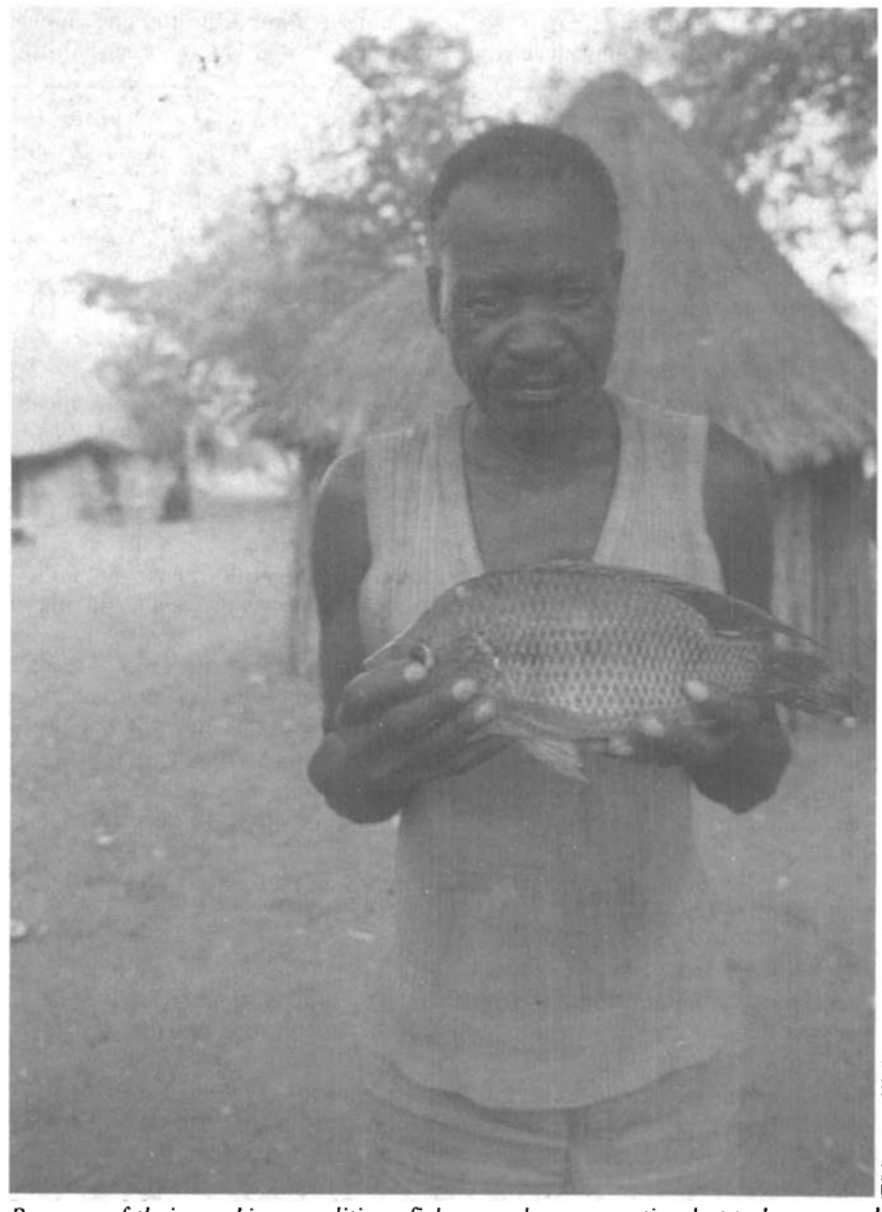

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Because of their working conditions fishermen have no option but to be exposed to risk from cholera in the aquatic environment.