Cholera and other vibrio-associated diarrhoeas*  

WHO SCIENTIFIC WORKING GROUP 1

In recent years, there have been major advances in knowledge of Vibrio species and related organisms that are responsible for diarrhoeal diseases, particularly V. cholerae O-Group 1 (epidemic strains), atypical V. cholerae O-Group 1, non-O-Group 1 V. cholerae (non-epidemic strains), V. parahaemolyticus, V. alginolyticus, and "Group F vibrios". This article reviews the important new information, and identifies gaps in our knowledge, on aspects such as the epidemiology and bacteriology of vibrios, environmental surveillance for V. cholerae O-Group 1, phage and vibriocin typing of V. cholerae, and cholera enterotoxin, and its relevance to pathogenesis, immunity, and vaccine development. In each of these areas priorities for further research are recommended.

The seventh cholera pandemic, which began nearly 20 years ago, is continuing to spread. Eight new countries were affected in 1978, and several previously affected countries in Asia and Africa experienced severe recrudescences and extensions of the disease, causing major problems for national health authorities. The pandemic has stimulated extensive and intensive research on cholera and related subjects during the last two decades. This research has made many significant contributions to knowledge of the etiology, epidemiology, pathogenesis, clinical management, and immune mechanisms of all acute diarrhoeas, and has made it possible to reduce cholera-related mortality to less than 1% in well-equipped treatment centres.

This article reviews significant recent information on the epidemiology and bacteriology of diarrhoeal diseases caused by Vibrio species and related organisms, draws attention to remaining gaps in knowledge, and recommends priority areas for further research. The related subject of vaccine development is also reviewed briefly.

TERMINOLOGY

The nomenclature and taxonomy of the genus Vibrio are in an uncertain state. Until recently, the name "Vibrio cholerae", and the term "cholera vibrio", were generally restricted to the organism causing epidemic cholera, and terms such as "non-agglutinating vibrios (NAGs)" and "non-cholera vibrios (NCVs)" were used rather ambiguously to describe either all vibrios, including the halophilic vibrios, that did not agglutinate in cholera O-Group 1 antiserum (polyvalent O-1 antiserum), or only the group of vibrios that were biochemically similar to the epidemic cholera strains. Uncertainty about which meaning authors gave these terms made communication between scientists difficult and impeded the development of knowledge about vibrios other than the epidemic strains. When taxonomists recently sought to clarify the nomenclature of the genus Vibrio, they unfortunately placed all vibrios that were similar biochemically and by DNA homology to

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the epidemic strain into one species: \textit{V. cholerae}. The epidemic strains were then classified as \textit{V. cholerae} O-Group 1, and the others as different serotypes of the same species.

It is extremely unfortunate that these other vibrios, many strains of which are non-pathogenic or of doubtful pathogenicity, should have been included in the species \textit{V. cholerae}, which to the public, microbiologists, physicians, and public health personnel has an implied epidemic potential. Another species name that did not already have a well-established and emotive meaning should have been selected. As will be shown below, there are important epidemiological and biological distinctions between the epidemic strains and these other vibrios. While, in the absence of a more satisfactory alternative, it was decided to use the nomenclature recommended by the taxonomists, with some modifications, in this article, it must be emphasized that this should not be considered as constituting approval of the nomenclature by WHO.

Thus, the following definitions will be used:

1. The epidemic strain will be referred to as \textit{V. cholerae} O-Group 1 (epidemic strains), or \textit{V. cholerae} O1.

2. As will be explained in more detail below, a few vibrio strains have been isolated that agglutinate in one or more lots of polyvalent O-1 antisera but are apparently non-pathogenic in that they do not produce enterotoxin in \textit{in vivo} and/or \textit{in vitro} assay systems. Some of these also have a few atypical biochemical properties. These strains will be referred to here as atypical \textit{V. cholerae} O-Group 1, or atypical \textit{V. cholerae} O1, in recognition that they share with the vibrios responsible for epidemic cholera an antigenic mosaic that should be recognizable by virtually any polyvalent \textit{V. cholerae} antisera of reasonable quality.

3. Organisms that are similar biochemically to the epidemic strains but do not agglutinate in polyvalent O-1 antiserum will be referred to as non-O-Group 1 \textit{V. cholerae} (non-epidemic strains), or non-O1 \textit{V. cholerae}. These organisms do not appear to be responsible for epidemics of severe diarrhoea, although they have been associated with individual cases and small outbreaks of diarrhoea, and some appear to produce cholera-like enterotoxin.

4. Other vibrios, such as \textit{V. parahaemolyticus}, \textit{V. alginolyticus}, and “Group F vibrios”, are clearly distinct species and will be so regarded in this article.

A summary of some of the characteristics of these groups is given in Table 1.

\section*{Epidemiological Significance of Vibrios}

\textit{Vibrio cholerae} O-Group 1

\subsection*{Global cholera situation}

The number of countries affected during the present pandemic has increased in two main phases: a gradual increase from 1961 until 1966, and a major upsurge in 1970, when countries in Africa were infected. Since then, the number of countries affected has levelled off, although the number of additional countries infected in 1978 (8) was the highest recorded in any one year since the extension of cholera to the African continent. A source of continuing concern is the fear that, if cholera should reach any of the countries of South and Central America, which are considered to be “receptive”, there could be another dramatic increase in the number of countries affected.

Since 1961, the El Tor biotype of \textit{V. cholerae} O1 has rapidly replaced the classical biotype so that, in the 1970s, the global cholera problem has been virtually exclusively caused by the El Tor biotype; however, rare and isolated cases due to the classical biotype do still occur, such as those reported from India in 1978 and 1979 and from Bangladesh in 1979 for
reasons that are not known. The El Tor biotype appears to have a greater “endemic tendency” than classical *V. cholerae* O1. Also, in infections with the El Tor biotype, there has been a higher infection-to-case ratio, making surveillance, outbreak investigation, and control potentially more difficult.

The strains causing the present pandemic were initially differentiated as the El Tor biotype because of their ability to haemolyse sheep erythrocytes. It was also shown that, unlike the classical biotype, they agglutinated chicken and sheep erythrocytes and were resistant to classical phage IV and to polymyxin B (50-unit disc)—the Voges-Proskauer reaction was not as reliable. As the pandemic has progressed, the proportion of strains isolated that are haemolytic has gradually decreased, and now for routine practical purposes El Tor strains are indistinguishable in this test from the classical biotype. Generally, however, these isolates still behave as El Tor in the other biotyping tests, although these tests need to be done with great care to give consistent results.

**The carrier state**

It is generally agreed that the true long-term carrier state is extremely rare in cholera. Occasional cholera patients—such as “Cholera Dolores” in the Philippines—become long-term carriers, but secondary outbreaks associated with such individuals have never been documented. It is not known if pre-existing inflammation of the gall-bladder can predispose to the carrier state, or if a normal gall-bladder can be infected. Either the biotype of *V. cholerae* O1 or the prior immune status of the host may be important in predisposing
to the carrier state; when Iran was newly infected with El Tor *V. cholerae* O1 in this pandemic and convalescent cholera patients with culture-negative stools were purged with magnesium sulfate, cultures of the last portion of purged stool were frequently positive. However, when the same procedure was carried out in convalescent cases in an endemic area, Bangladesh, at a time when the classical biotype still prevailed, the cultures were usually negative.

**The infective dose in man**

It is becoming increasingly evident that the number of vibrios required to cause symptomatic infections is lower than previously believed. When a strain of the classical biotype was administered to adult volunteers in water, the ID$_{50}$ was $10^8-10^9$ vibrios. Prior administration of sodium bicarbonate reduced the infectious dose to approximately $10^4-10^5$. However, in recent studies using an El Tor strain, administration of $10^3$ vibrios with bicarbonate resulted in symptomatic infection in 4 out of every 6 volunteers challenged. It is clear from these and other epidemiological and clinical studies that gastric acidity is a major factor in host resistance, the disease being more common in persons with hypochlorhydria. Susceptibility may also be increased by more rapid gastric emptying following intakes of large amounts of food and water. Volunteer studies with the El Tor biotype have established that the infective dose is lower when organisms are administered with food than when they are given in a small amount of water. It is not yet known if food itself acts by neutralizing gastric acid or if vibrios are protected from acid by adhering to food particles. One related observation is that vibrio adhesion to chitin (crabshell) particles enhances vibrio survival in an acid environment. It is thus not unreasonable to assume that a dose as low as $10^2$ or $10^3$ viable vibrios may be able to cause symptomatic infections, and one study of cholera in rural Bangladesh suggests that such doses may lead to such infections under natural conditions.

**Mechanisms of transmission**

*Vibrio cholerae* O1 can survive and even multiply in various foods and in water. In general, vigorous efforts have been needed to determine the mode of transmission in cholera outbreaks, especially in newly infected areas (see page 371). In some instances, epidemics have been aborted by rapid rational and instinctive measures to control suspect vehicles such as the immediate chlorination of water supplies.

Man is the major source of infection and the disease is usually spread geographically by symptomatic and asymptomatic persons whose evacuations reach and contaminate food and water. The vibrios are probably also transported between adjoining areas by moving bodies of water that have been contaminated with infected faeces. The possibility that other reservoirs of infection, such as infected shellfish or coastal waters, can maintain vibrios for prolonged periods cannot, however, be excluded (see page 364).

There have been a number of other important epidemiological findings in recent years. For example, in Bahrain, the incidence of cholera has been found to be higher in bottle-fed than in breast-fed infants. It has been observed in both Calcutta, India, and in rural Bangladesh that the seasonal incidence of cholera can change dramatically over a short time. The disease used to be most common in the summer in Calcutta and in the early winter in Bangladesh; now in both places it is most frequent in the autumn. Recent studies in rural Bangladesh have also shown that access to adequate supplies of pure drinking water does not necessarily lessen the incidence of cholera in some areas, especially those where sanitation is poor and impure water is used for other purposes (e.g., cooking).
Antibiotic resistance and treatment

An epidemic of cholera which began in Tanzania in October 1977 offers a well-documented example of the development of antibiotic resistance among V. cholerae O1. All isolates during the first month following recognition of the disease were fully sensitive to tetracycline, but after 5 months of extensive therapeutic and prophylactic use of the drug (1788 kg during the first 6 months) 76% of isolates were found to be resistant. The resistance was found to be mediated by at least two closely related plasmids belonging to the C incompatibility group, one of the few groups of enteric plasmids that can exist stably in V. cholerae O1. Both the plasmids identified confer multiple antibiotic resistance. This appears to be one of the first examples of an independent outbreak of V. cholerae O1 strains carrying resistance-mediating plasmids. Despite a substantial reduction in the use of tetracycline after the detection of this phenomenon, resistance continues to be common among V. cholerae O1 isolates in Tanzania. The possible spread of resistant strains to neighbouring countries is a matter of grave concern. The events in Tanzania should lead to a reappraisal of the use of antibiotics, particularly as a mass prophylactic measure, in the control of cholera epidemics.

Atypical Vibrio cholerae O-Group 1

Before 1961, when the present cholera pandemic began, diarrhoeal illness caused by the El Tor organism was thought to occur only in Indonesia. However, El Tor vibrios had been isolated from surface waters in the Eastern Mediterranean area and India long before that date. These El Tor “water vibrios” were subsequently isolated from surface and well-waters both in cholera-free areas and in endemic areas during seasons when there was no cholera. Some of these isolates were studied extensively during the early 1960s as possible vaccine strains and were found to produce little if any cholera enterotoxin.

During the 1970s, atypical V. cholerae O1 strains of this kind have been isolated in at least eight areas from man and/or other sources. The available information about them is summarized below.

Recent isolates

(a) In Guam in 1974, during an investigation of a small outbreak of cholera caused by V. cholerae El Tor, serotype Ogawa, 7 strains of atypical V. cholerae O1 were isolated from sewage (1), storm drains (4), a river (1), and a bay (1). Despite extensive testing of persons with diarrhoea as part of the cholera investigation, no atypical strains were isolated from human subjects.

(b) In April 1977, a 61-year-old truck driver in Alabama, USA, suspected of having acute cholecystitis of 12 days’ duration, underwent a cholecystectomy. V. cholerae El Tor, serotype Inaba, was isolated from a culture of the bile from the gall-bladder, obtained at surgery. The patient had not had a recent diarrhoeal illness, and, except for a brief trip into Mexico 30 years previously, he had not travelled outside the USA. He had an Inaba vibriocidal titre of 1/320. The 3 other members of his family were culture-negative and did not have elevated vibriocidal antibody titres. None of the 4 had serum antitoxic antibodies detectable by the rabbit-skin assay.

(c) In Bangladesh in 1977, an attempt was made to detect atypical V. cholerae O1 strains in man and the environment. Of 82 isolates from the environment, one was non-toxigenic, while 1275 clinical isolates were all toxigenic.

(d) In 1977, one of 65 isolates of so-called Vibrio cholerae from Chesapeake Bay, on the eastern coast of the USA, proved to be atypical V. cholerae O1. At least one large hospital
near Chesapeake Bay has been using TCBS agar\(^a\) routinely for all stool cultures for at least 5 years without detecting any isolates of *V. cholerae* O1.

(e) Since 1974, Brazil has been carrying out routine surveillance of sewerage systems for *V. cholerae*, using Moore swabs. All tests were negative until May 1978, when 2 strains of *V. cholerae* O1 were isolated from sewage from Santos, a city near São Paulo. Although sewage surveillance was then intensified and TCBS agar introduced for culturing stools from persons with diarrhoea, no more *V. cholerae* O1 strains were detected. In October 1978, 2 strains of *V. cholerae* O1 were detected in sewage from the Rio de Janeiro sewerage system. There again, no human infections were identified.

(f) At least 11 sporadic cases of cholera caused by *V. cholerae* O1 El Tor Inaba occurred in Louisiana, USA, in 1978. In the course of extensive culturing of specimens from persons with diarrhoea, raw crabs, shrimps, oysters, sewage, and surface water, a few strains of *V. cholerae* El Tor Inaba were found; all were toxigenic and of the same phage type.

During 1979, surveillance of cases of diarrhoea and of sewerage systems throughout the southern half of Louisiana continued, and oysters from commercial distributors were routinely cultured. As of 17 September 1979, 11 apparently atypical *V. cholerae* O1 strains had been isolated, none of which had the same phage type as the 1978 toxigenic strains. Of these strains, one came from a canal with high faecal coliform counts, and 8 from oysters taken from supposedly clean areas. The remaining 2 isolates were from New Orleans and may represent the same strain, being identical in every laboratory test performed, including phage sensitivity. One of them was isolated from a large, necrotic ulcer on the leg of a vagrant. The man flushed the soiled bandages down his toilet daily, and the second isolate was from a sewage line draining an area that included the patient’s toilet. This was the only isolate of *V. cholerae* O1 from New Orleans sewage during a period of more than 8 months of routine surveillance with Moore swabs.

(g) For 5 weeks during 1977, atypical *V. cholerae* O1 Ogawa strains were continuously isolated from a brackish agricultural drainage ditch in England where the possibility of sewage contamination was considered to be negligible.

(h) In May 1978, *V. cholerae* El Tor Inaba was isolated from a river near the port of Yokohama in Japan. Environmental studies revealed the source as the septic tank of a hospital used for the disposal of artificial kidney dialysate. No cholera cases or carriers were found. Tests of the isolates to date indicate that they are non-toxigenic.

Finally, workers in the USSR have reported finding that 270 strains of *V. cholerae* O1 El Tor, isolated over 11 years from various sources, could be subdivided into 3 groups by using the suckling rabbit assay. One group, which was highly enterotoxigenic (choleragenic), failed to lyse sheep erythrocytes. Another group was haemolytic and caused death in test animals without choleraigenic effects. The third group was also haemolytic but was avirulent in suckling rabbits, even when high doses were administered.

It can be seen from this review that atypical *V. cholerae* O1 have been isolated primarily from environmental sources and that, despite extensive searches for these strains in the stools of diarrhoea cases (e.g., in Brazil, Guam, and Louisiana) during and shortly after the time when atypical strains were being isolated from environmental sources, to date such isolates have been associated in man only with extraintestinal disease (cholecystitis, wound infection). It is also evident that these atypical *V. cholerae* O1 strains have a global distribution (Asia, the Pacific Islands, North America, Europe, and the Eastern Mediterranean). There is also some suggestion that they may be free-living: the strains in England and those from oysters in Louisiana appear to have come from areas without sewage contamination.

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\(^a\) Thiosulfate-citrate-bile-salts-sucrose agar.
**Bacteriological characteristics**

Difficulties have been encountered in serotyping some of the strains, and particularly in using polyvalent antisera. Variable results were obtained with four different batches of polyvalent (O-Group 1) antiserum.

The biochemical reactions of some strains were also atypical. The majority of the strains from Guam fermented sucrose slowly. Seven out of 8 isolates from oysters in Louisiana did not ferment mannitol; by the criteria of Hugh & Sakazaki, in fact, these strains were not *Vibrio* species, despite being typical in other respects.

Although all the recent strains were of the El Tor biotype, many had atypical biotype patterns. Those from Brazil were all sensitive to polymyxin B. The strain from Alabama and 3 strains from Louisiana were sensitive to classical phage IV in one laboratory; however, another laboratory found the Alabama strain to be resistant to this phage. The results of the chicken red blood cell agglutination and Voges-Proskauer tests also varied from strain to strain.

Most of these atypical strains were not markedly sensitive to any of the classical or El Tor phages. They were almost uniformly resistant to phages 13, 14, 16, and 24 of the Public Health Laboratory, Maidstone, England. In contrast, all known *V. cholerae* O1 associated with human infections have, so far, been sensitive to one or more of these phages (see page 367).

**Pathogenicity**

With the exception of the mouse lethal assay (intraperitoneal injection of living cells), which is of doubtful relevance, the results of tests for pathogenicity on the atypical strains have been almost uniformly negative. In the rabbit ligated ileal-loop assay, the only suggestion of activity occurred in a few cases in which live organisms were used; culture filtrates (unconcentrated) have all been negative. Some of the strains have produced haemorrhagic lesions in the rabbit intracutaneous assay.

In the Y-1 adrenal cell assay, the two strains from Santos, Brazil, gave some weakly positive reactions, but they were non-toxigenic in the ELISA and rabbit intracutaneous assays. They have been reported to produce the A but not the B portion of cholera toxin. Both strains have been given orally to volunteers. Strain 1074–78 was given to 7 volunteers in a dose of 10⁶ organisms with bicarbonate: none became ill and their stools were culture-negative. Strain 1196–78 was given to 8 volunteers in a dose of 10⁶ with bicarbonate: the stools of 6 were culture-positive and none became ill. Sera from these volunteers have not yet been tested to determine whether they developed antitoxic antibodies. Ten isolates from each of the 6 volunteers who excreted strain 1196–78 (a total of 60 isolates) were tested in the adrenal cell assay. Only one isolate was toxigenic at low titres, but a clone of this isolate was found to be non-toxigenic. Four of those who excreted the organism were subsequently challenged with a toxigenic *V. cholerae* O1 strain from Bahrain, and all became ill. Thus, colonization by the Brazilian strain did not confer protection. This observation and the previous finding that oral administration of living hypotoxigenic or killed cholera vibrios affords protection against subsequent challenge with virulent *V. cholerae* suggest that there may be significant differences in somatic antigens between the atypical *V. cholerae* O1 and toxigenic O1 strains. Strain 1196–78 has also been given in a higher dose (10⁶ organisms) with bicarbonate to 5 volunteers: it was isolated from the stools of 2 of the 5, but none became ill.

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*c* There are no internationally accepted and standardized methods for measuring pathogenicity. Such factors as inoculum size, time of incubation, source of laboratory animals, and definition of a positive response, which can profoundly affect the observed results, may vary from laboratory to laboratory. Thus, the results summarized here should be interpreted with caution.
Non-O-Group 1 *Vibrio cholerae*

**Clinical features**

These organisms have been associated with outbreaks and sporadic cases of gastrointestinal illness, but their clinical features cannot be characterized with any confidence. From the descriptions of sporadic cases, one cannot be certain that the organism isolated was always the cause of the illness. In the reported outbreaks, the clinical features varied, possibly reflecting different characteristics of the responsible strains. In general, the patients had diarrhoea, nausea, and vomiting. Some had fever and abdominal cramps, and a few had blood or mucus in their stools. The illness usually lasted less than 3 days.

In a comprehensive study conducted in Bangladesh, patients yielding isolates that produced a cholera-toxin-like enterotoxin had more severe illness than those yielding non-toxigenic isolates. Some significant rises in serum agglutinating titres against the homologous strain were demonstrated in both groups, and significant antitoxin titre rises occurred in some patients with toxigenic isolates.

Extraintestinal non-O1 *V. cholerae* infections have been reported in debilitated or immunosuppressed hosts, and some have been fatal.

**Epidemiology**

Non-O1 *V. cholerae* has been isolated from the stools of persons with diarrhoeal illnesses in Asia (Bangladesh, Hong Kong, India, Malaysia), Africa (South Africa, Sudan), Europe (Bulgaria, Czechoslovakia, Federal Republic of Germany, Hungary, Sweden, United Kingdom, USSR), North America (Mexico, USA), South America (Brazil) and on board ships and aircraft. The frequency of infections with these organisms in these areas has not been comprehensively studied. The organisms could undoubtedly be isolated in other countries where investigations have not so far been carried out. Large epidemics and pandemics like those caused by *V. cholerae* O1 have not been reported.

Little is known about the seasonality of non-O1 *V. cholerae* disease. In the USA, most infections occur during the warmer months. In Bangladesh, most of the cases occur during the spring and summer before the annual increase in *V. cholerae* O1 cases in the autumn.

Transmission is probably exclusively by contaminated food and water. Studies have shown that non-O1 *V. cholerae* can multiply in a variety of foods. In the USA, patients frequently have a history of consumption of molluscs, especially raw oysters, within the 48 hours preceding the onset of diarrhoea. In outbreaks in Czechoslovakia and Australia, the vehicle of transmission was food (potatoes, and an egg and asparagus salad, respectively). The incubation periods in these outbreaks were 20–30 hours, and 5–37 hours. In a large outbreak in the Sudan in 1968, polluted well-water was responsible; cases ceased to occur 4 days after the suspect well had been closed. Despite the large number of exposed persons, no secondary cases were observed and no person-to-person transmission was evident.

Non-O1 *V. cholerae* strains have been found to be widely distributed in the environment wherever they have been looked for, namely in some countries of Europe and in the USA. They have been found in sewage, sewage-contaminated surface water, estuarine waters (both with and without sewage-contamination), seafoods, and animals. The organisms found in ecological studies carried out in the Federal Republic of Germany, the United Kingdom, and the USA were generally in brackish surface waters (rivers, marshes, bays, and coastal areas), were more numerous during the warmer months, and were not associated with sewage contamination. They are thus usually considered as aquatic organisms and widely thought to be free-living in the environment. However, it is not known whether free-living strains cause disease in man; pathogenicity may be restricted to strains adapted to the human intestine.
Pathogenicity

Using a battery of assays (rabbit ileal loop, infant rabbit, rabbit skin permeability, suckling mouse, Chinese hamster ovary cell) four patterns of biological activity have been observed in non-O1 V. cholerae. Of potential value for describing, classifying, and comparing these organisms, they are: (1) production of a cholera-toxin-like enterotoxin; (2) production of a heat-stable toxin; (3) "enteritis" (positive infant rabbit and/or positive ileal loop assay with use of whole bacterial culture, without evidence of production of either toxin); and (4) no activity in any of these assays. In the study done in Bangladesh (see above), 98% of 43 strains from patients with diarrhoeal disease showed some activity in at least one of these assays, while only 33% of 18 strains isolated from surface waters with low coliform counts did so. Isolates from outbreaks in the Sudan and Czechoslovakia have been found to produce a cholera-toxin-like enterotoxin, and studies in the USSR and Japan have strongly suggested that some strains of non-O1 V. cholerae produce other toxins. Despite these results, it must be borne in mind that there is no assay (other than human feeding experiments) or group of assays that can reliably determine if a given strain is a potential pathogen for man.

Serotyping

Two serotyping systems for non-O1 V. cholerae are in use: one developed by Shimada & Sakazaki and the other by Smith. Both systems are based on the O antigen (somatic antigen) and in both the classical and El Tor biotypes of V. cholerae are designated O-group 1. By 1979, the system developed by Sakazaki et al. included 60 serotypes, while 72 serotypes had been recognized by Smith. In spite of the differences in the methods used to prepare antisera and to perform agglutinations, many serotypes correspond in the two systems, although each system includes some that are unique. Neither system has so far determined any marked differences between the serotypes of isolates from human and non-human sources or in their diarrhoeagenic potential. The United States and Japanese Cholera Panels of the United States–Japan Cooperative Medical Science Program are currently attempting to develop a uniform serotyping system.

In the USSR, serotyping has been attempted with 2008 strains of non-O1 V. cholerae isolated from human and environmental sources between 1968 and 1975 in 18 territories of the country. Only 40.5% of strains from humans and 16% of strains from the environment could be serotyped by the Sakazaki system. The investigators demonstrated 15 serotypes that are not included in the Sakazaki system. Sakazaki serotype 5 appeared to be the predominant type in persons with acute gastrointestinal disease, whereas serotype 8 predominated in the strains from environmental sources.

Vibrio parahaemolyticus

Clinical features

V. parahaemolyticus, a halophilic marine vibrio, was first recognized as a cause of food poisoning in Japan in the early 1950s. Two clinical syndromes have been described. In the one more commonly observed, the cardinal manifestation is watery diarrhoea, although abdominal cramps, nausea, vomiting, and fever may occur. The other is a dysenteric syndrome which has been described in several countries; in Calcutta, for example, 60% of the reported cases have had dysentery. In the watery diarrhoea syndrome, the modal incubation period is 15 hours. However, in some cases of the dysenteric syndrome, the

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incubation period has been reported to be shorter (as short as 2.5 hours). The reason for this apparent difference has not been explained. In both syndromes the illness is usually self-limited, with a median duration of about 3 days. Although severe cases of *V. parahaemolyticus* enteric infection requiring hospitalization and even causing death may occur, the illness is usually of mild or moderate severity.

*V. parahaemolyticus* wound infections have also been reported from Australia, Canada, and the USA in persons with wounds exposed to sea-water.

**Epidemiology**

During the last decade, *V. parahaemolyticus* enteric infection has been reported from North America (USA), Central America (Panama), Africa (Togo), Europe (Romania, United Kingdom, and USSR), and Asia (Bangladesh, India, Indonesia, Japan, Korea, Malaysia, Philippines, Singapore, Thailand, Viet Nam). The frequency of isolation of the organism from diarrhoea cases in different countries varies widely: India (Calcutta), 11%; Indonesia, 2.6–3.7%; Thailand, 10.7%; Viet Nam, 8.5–15%; and Korea, 1.5%. In Japan, about 24% of reported cases of food poisoning are attributed to *V. parahaemolyticus*. In many countries the extent of the disease is unknown because most laboratories do not use culture media, like TCBS, that are appropriate for isolating the vibrio from stools.

Marked seasonality of the disease has been noted in several countries, most of the cases occurring during the warmer months. This may reflect both greater opportunities for the multiplication of *V. parahaemolyticus* in unrefrigerated foods and an increased prevalence of the vibrio in the environment. However, in Calcutta, where the ambient temperature varies relatively slightly, there is little seasonal variation in incidence. Over 65% of the cases in Calcutta occur in females, and most patients are over 15 years of age. Although asymptomatic infections have been found, there have been no reports of long-term carriage of the organism.

Enteritis due to *V. parahaemolyticus* appears to be transmitted exclusively by food. The vehicle of transmission is usually seafood, although other types of food contaminated by raw seafood or surface water are also thought to play a part. In Calcutta, *V. parahaemolyticus* has been found in stools, food, and water in strictly vegetarian households, and in many cases there is no history of seafood consumption or other exposure related to the sea. The generation time of *V. parahaemolyticus* is reported to be as short as 9 minutes under ideal conditions, enabling the organism to multiply very rapidly in mishandled foods, rapidly reaching the rather large infective dose: the ID$_{50}$ has been determined by volunteer studies to be about $10^8$–$10^7$ organisms in persons given antacids. The growth of *V. parahaemolyticus* is inhibited at temperatures below 15 °C and the organism tends to die out at lower temperatures. It is quite sensitive to high temperatures, too, succumbing after exposure at 65 °C for 10 minutes.

*V. parahaemolyticus* is part of the normal flora of estuarine and other coastal waters throughout most of the world. It has been isolated from seawater, sea mud, or seafood in Asia (Hong Kong, Japan, Korea, Singapore, Sri Lanka), North America (Canada, USA), Oceania (Australia, Hawaii, New Zealand), Africa (Togo), and Europe (Baltic Sea, Black Sea, Mediterranean Sea, North Sea, Denmark, Greece, Italy, Netherlands, Spain, Turkey, United Kingdom, Yugoslavia). It has been isolated from freshwater and fresh fish, crab, and shrimp in India. In estuarine waters in temperate regions, it has been observed that *V. parahaemolyticus* passes the winter in sediment, is released from the bottom in the spring and becomes attached to zooplankton, and then proliferates as the water temperature rises.

**Pathogenicity**

Almost all *V. parahaemolyticus* isolates from patients with diarrhoea, but only about 1% of isolates from seafoods and seawater, are Kanagawa-positive on Wagatsuma agar.
However, it is not certain that all Kanagawa-positive strains are pathogenic. The Kanagawa reaction is caused by a heat-stable direct haemolysin with a relative molecular mass (RMM) of approximately 42,000. The haemolysin is cytotoxic to human FL cells in cell culture, and is cardiotoxic for mice. Although minor changes have been described in the electrocardiograms of acutely ill patients, the clinical importance of the heat-stable haemolysin is not known.

Kanagawa-positive and Kanagawa-negative organisms have been studied intensively, and their activity in a number of laboratory assays differs greatly. Kanagawa positivity is associated with penetration of the intestinal epithelium of infant rabbits, rapid cytotoxicity in HeLa cell cultures, rapid adhesion to HeLa and human fetal intestinal cells, and production of a heat-labile factor that produces a cholera-toxin-like reaction in Chinese hamster ovary (CHO) cells.

Despite extensive study of the pathogenic mechanisms of *V. parahaemolyticus*, it is still not known how the two gastrointestinal syndromes described above are produced.

**Serotyping**

By 1976, 12 O antigens (heat-stable somatic antigens) and 59 K antigens (capsular or envelope antigens) had been identified. So far, there has been no strong, worldwide association of any particular serotypes with the Kanagawa phenomenon or with illness in man, although in Calcutta one-third of the cases have been associated with the isolation of serotype O1 K56. It thus appears that serotyping may be useful for epidemiological investigations but not for diagnostic purposes.

**Group F (EF6) vibrios**

A group of vibrio-like organisms designated Group F by the Public Health Laboratory, Maidstone, England, probably constitutes a new species (Table 1). These organisms are identical to organisms designated as Group EF6 by the US Center for Disease Control. They are often mistakenly identified as *Aeromonas*, but can be distinguished from the latter by their sensitivity to the vibriostatic compound 0/129 (150-μg disc) and their ability to grow in a 60 g per litre solution of sodium chloride. Group F vibrios can be divided into two biotypes: biotype 1 includes only anaerogenic strains of clinical and environmental origin, while strains of biotype 2 are generally aerogenic and have only been found in the environment. Biotype 1 strains have so far been isolated from patients with diarrhoea in Bahrain, Bangladesh, Egypt, India, Indonesia, Iran, Iraq, Jordan, Kenya, Philippines, Saudi Arabia, Spain, United Republic of Tanzania, and Tunisia.

The clinical features and epidemiology of disease associated with isolates of this group of organisms have not yet been well defined, but some information is available from Bangladesh where, in 1976–77, there was an increase in isolations of Group F organisms from patients attending a rural treatment centre. Prior to and after this period, there were very few isolations of the organism. About half of the isolates were from children below 5 years of age. In studies of family members of infected persons in Dacca, Group F organisms were found in the stools of less than 1%. The clinical symptoms of the cases were cholera-like, except that some persons had blood and mucus in their stools, abdominal pain, or fever. No agglutinating antibodies against the homologous organisms were detected. Nine isolated strains were tested at the US Center for Disease Control and found negative in the adrenal cell and suckling mouse assays, and in the Serény test. However, another institution has reported that whole cultures and culture filtrates of most of the strains tested produced fluid accumulation in ligated rabbit ileal loop, regardless of their source of isolation (faeces, sewage, or environment).
Despite the report from Bangladesh of a noteworthy increase in the numbers of Group F (EF6) vibrios in the stools of patients with diarrhoea, it must be concluded from the available epidemiological and laboratory data that it is uncertain at present whether they are diarrhoea-producing pathogens.

Other vibrio species and related organisms

Other vibrio species occasionally isolated from man—*V. alginolyticus*, *V. metschnikovii* (enteric Group 16), and *V. vulnificus* (lactose fermenting "vibrio")—are not believed to cause diarrhoeal illness in man. *V. anguillarum* has not been isolated from man, but is an economically important pathogen of salmonids and other marine fish; virulent strains have been shown to harbour plasmids not found in avirulent strains. *Aeromonas hydrophila* and *Plesiomonas shigelloides* have been isolated from the stools of children and adults with diarrhoea, and a number of workers think they may have played a causative role. Strains of *Aeromonas* have been shown to produce various toxins, and both cultures and filtrates cause accumulation of fluid in rabbit ileal loops.

ENVIRONMENTAL SURVEILLANCE FOR *V. CHOLEREA* O-GROUP 1

In the last decade there has been considerable interest and research in the behaviour of *V. cholerae* O1 in the environment and the use of environmental surveillance as a means for early detection of the organism in a non-infected area. Recent information in this field is summarized below.

Survival of *V. cholerae* O-Group 1 outside the human intestine

Most evidence suggests that *V. cholerae* O1 depend on the human intestinal tract as their primary multiplication site and reservoir and that in aquatic environments they are alien and ultimately eliminated. Several apparent exceptions indicate that this may not always be the case:

(a) In the cholera outbreak in Louisiana during 1978 (see page 358), the organism incriminated was a haemolytic El Tor Inaba strain with the same unusual phage sensitivity pattern as the haemolytic El Tor Inaba strain isolated from a cholera case in Texas in 1973. Although cases could easily have been missed, there is no evidence that other human cholera infections occurred in the intervening years, which suggests that the organisms may have persisted in the environment.

(b) In Australia, since 1977, El Tor Inaba vibrios have been isolated repeatedly over a 25-month period from a freely flowing river. The peak incidence coincides with the warmest part of the year. No human source of contamination has been found, and only 2 human infections have been detected, both before discovery of the vibrios in the water. The organism has also been isolated, in 2 out of 60 instances, from the gut contents of common sea mullets taken from the river. Isolates that have been tested appear in general to be toxigenic, though a number fail to elicit a positive ileal loop or are negative in the Y-1 adrenal cell assay.

(c) In the USSR, following a cholera outbreak in Astrakhan in 1970, *V. cholerae* O1 El Tor were isolated from two small water basins near the Volga river for periods of up to 14 months. These waters were not subjected to known human faecal contamination. In 1975,
El Tor vibrios were isolated from sulfurous spring waters in the region. The source of contamination may have been tourists visiting the region from cholera-infected areas, but this was not proved.

In addition, extensive environmental surveillance carried out in Bangladesh to identify an extraintestinal habitat for *V. cholerae* O1 revealed that the organism is sometimes associated with the root surfaces of plants, particularly the water hyacinth (*Eichhornia crassipes*). Laboratory studies carried out in isolated tanks of fresh pond-water indicated that El Tor vibrios and many other heterotrophic bacteria are concentrated on the root surfaces of these plants within a few hours of immersion. Vibrios adhering to plants remained viable longer than those left free in the water.

**Methods used in environmental surveillance**

**Sampling**

The use of Moore swabs has been shown to be effective in monitoring flowing water and sewers for *V. cholerae*, and it is the most sensitive technique available when counts are less than 1 per litre. In still water, it is usually necessary to collect a number of water samples of 1 litre or more. Such samples should be enriched directly or after filtration through Celite. As surface plants and plankton have sometimes yielded *V. cholerae* O1 when the surrounding water has not, the sampling of such aquatic organisms may increase the rate of vibrio detection.

It is important that samples should be processed as soon as possible after collection and maintained at ambient temperature during transport to the laboratory. Enrichment culture can be started in the field. *V. cholerae* O1 isolates from water sometimes appear to have suffered sublethal injury that increases their sensitivity to cold shock. If samples must be cooled for transport, still-air cooling (no direct contact with coolant) should be employed.

**Enrichment**

Alkaline peptone water (APW) without NaCl is the best available enrichment medium, though it permits the growth of too many competitors to be ideal. Cultures can be streaked at 6–8 hours and at 18–20 hours or, alternatively, it may be better to start a subculture after 6–8 hours and then streak the original culture and the subculture after overnight incubation. The 6–8 hour incubation can be carried out at 35–37 °C or at ambient temperature; the optimum temperature for overnight incubation is 35–37 °C. Various other enrichment broths have been suggested, but they present no real advantage.

**Plating**

TCBS agar is recommended for plating enrichment cultures. The efficiency of this medium can vary widely from one brand or lot to another, and the Eiken brand appears to be the most reliable. Environmentally-stressed toxigenic *V. cholerae* O1 grow very poorly on this highly selective medium, and direct plating of water samples on to TCBS is not recommended.

**Identification**

An efficient procedure for identifying *V. cholerae* O1 is to pick up smooth yellow colonies (atypical O1 and non-O1 strains occasionally form green colonies) from TCBS and transfer them to gelatin neopeptone agar containing no NaCl. Isolates that grow and produce gelatinase zones should be tested for oxidase reaction and slide agglutination in
polyvalent *V. cholerae* O1 antiserum and, if positive, in type-specific Ogawa and Inaba antisera. Non-agglutinating isolates can be transferred to Kligler's iron agar and tested in addition for lysine and ornithine decarboxylase and arginine dihydrolase production in order to screen for non-O1 *V. cholerae* (see Table 1). Some difficulties in obtaining typical agglutination reactions with colonies selected from TCBS agar have been reported.

**Quantification**

Quantification of *V. cholerae* O1 in water samples is best done by a 3-tube MPN* procedure using APW and follow-up streaking on TCBS agar. Membrane filtration and incubation of the membrane on TCBS is not satisfactory. Pre-incubation of membranes on starch agar for 2–3 hours before transfer to TCBS has been found to be as efficient as the MPN procedure only when assaying water with high (≥10³/litre) concentrations of *V. cholerae* O1, or water with low turbidity and few competitors.

The extent to which counts are affected by the organism's adherence to particulate matter has not been studied sufficiently. Blending has been shown in some cases to increase the apparent concentration of *V. cholerae* O1 in water samples.

**The problem of overgrowth of V. cholerae O1 by non-O1 V. cholerae**

There is no selective procedure available that permits the outgrowth of *V. cholerae* O1 from a population of non-O1 strains. If low numbers of O1 strains are present (as is frequently the case) in a sample with a large number of non-O1 strains, the O1 strains can be unrecoverable in practice and thus exist for long periods of time as a 'silent' population.

**The role of environmental surveillance in cholera control**

Sewer surveillance using only Moore swabs (in South Africa and Louisiana, USA) and sampling of pooled night-soil and of latrines (in Hong Kong) have proved to be sensitive indicators of the presence of *V. cholerae* O1 in a community. In cholera-affected areas served by sewerage systems, sampling with Moore swabs can be a cost-efficient adjunct to diarrhoeal disease surveillance for the monitoring of *V. cholerae* O1. In cholera-free areas, routine surveillance should be limited to common sewer lines. If *V. cholerae* O1 are detected, additional Moore swabs can then be placed in branch lines to assist in locating the source. In areas where cholera is sporadically present, surveillance at sewage disposal sites may be the least expensive and least difficult way to detect the occurrence of cholera in the community.

**PHAGE AND VIBRIOCIN TYPING OF V. CHOLERAЕ**

Although this has also been an area of expanding interest, the information available, which is summarized below, is still limited.

**V. cholerae** O-Group 1

It is agreed that a phage-typing scheme would be very helpful for epidemiological studies of toxigenic and atypical human and environmental *V. cholerae* O1 isolates. The practical

*fn* Most probable number.
value of the original scheme of Basu & Mukerjee is limited because recent isolates have fallen into only 3 types. In fact, the only published work demonstrating the epidemiological use of this scheme has been that describing an outbreak of cholera in Togo, in which it was used along with the scheme of Gallut & Nicolle.

Recently, however, an expanded phage-typing scheme has been developed at the Public Health Laboratory (PHL), Maidstone, England, where a collection of freeze-dried control cultures has been started. A number of phages have been screened and 14 have been shown to be useful for typing purposes: Mukerjee's classical phages I to IV; Basu & Mukerjee's phages 4 and 5; Nicolle's β; 4996, 13, 14, 16, and 24 isolated in Bangladesh; and 32 and 57 which are derived from Basu & Mukerjee's 3 and 5, respectively. Tests of 1135 strains revealed 25 patterns of sensitivity to these 14 phages (Table 2). The types are numbered 1–25 for the purposes of this paper. They appear to be reasonably stable, although occasionally strains sensitive to only one or two phages may give variants of wider sensitivity on repeated subculturing. This does not appear to be a problem with freshly isolated strains and is not significant enough to interfere with the use of the scheme for routine typing.


### Table 2. Phage typing of V. cholerae O1

<table>
<thead>
<tr>
<th>Basu &amp; Mukerjee type</th>
<th>Type number</th>
<th>Sensitivity at RTD to bacteriophage</th>
<th>No. in type</th>
<th>% in type</th>
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<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
<td>III(^b)</td>
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<tr>
<td>Total</td>
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</table>

+ : Sensitive; V: variable.

\(^a\) Routine test dilution.

\(^b\) Ill gives variable weak reactions with many El Tor strains in the common types; these have not been shown.

\(^c\) Occasionally may give a weak reaction of just a few plaques.
Almost all (99%) of the strains were typable, but 64% again fell into three types (11, 6, and 7). The selection of strains was biased: some countries, notably Bangladesh, India, and Indonesia, were much more heavily represented than others. Included in the 1135 strains were 114 which had previously been shown to be of Basu & Mukerjee's type 4 and sensitive to $\beta$. They were selected because it was known from earlier studies that this was the most common type, and it was hoped that they could be subdivided with the new phages. In fact, all except one of them fell into type 11. The predominance of a few types, in particular type 11, may be explained by the fact that the majority of the typing phages were isolated in one endemic area (the Ganges Delta). Phages isolated from the environment have so far proved to be of greater value than those from lysogenic strains. It therefore seems that new typing phages should be sought in the environment in other cholera-endemic areas.

The PHL Maidstone phages are of considerable promise as epidemiological tools, as the following three examples show:

(a) During the Portuguese outbreak in 1974, type 16 predominated in Portugal and in southern African states, but it has not yet been detected in other Mediterranean countries. This supports the belief that cholera was introduced into Portugal from southern Africa.

(b) The $V.\text{cholerae}$ O1 strains isolated from humans and the environment in Louisiana, USA, during 1978 were all of type 17, as was the strain isolated in Texas in 1974 (see page 364); this type has not been detected in any other country.

(c) Environmental $V.\text{cholerae}$ O1 isolates known to be atypical (non-toxigenic) and/or not associated with clinical cases have so far all been resistant to phages 13, 14, 16, and 24. Sensitivity to one or more of these phages may thus be an indication of potential pathogenicity.

In addition to this work, two groups of Russian workers have also isolated new phages active against $V.\text{cholerae}$ O1 strains which may be useful for typing. One of these groups, working in conjunction with the National Institute of Cholera and Enteric Diseases, Calcutta, India, divided O1 strains into about 20 types using Mukerjee's classical phages, Basu & Mukerjee's El Tor phages, Nicolle's $\beta$ phage, and 3 new phages.

Non-O-Group 1 $V.\text{cholerae}$

A scheme developed in Calcutta for phage-typing non-O1 $V.\text{cholerae}$ has not been widely applied and appears to have fallen into disuse. About 15% of 433 non-O1 strains recently typed with the PHL Maidstone phages were sensitive to at least one of these phages. The sensitivity patterns for non-O1 strains and some atypical (non-toxigenic) $V.\text{cholerae}$ O1 strains were the same. Sixteen out of 54 non-O1 strains examined were lysogenic. Since some of these phages are also active with $V.\text{cholerae}$ O1 strains, non-O1 strains may be a source of new typing phages for O1 strains.

Vibriocin typing of $V.\text{cholerae}$ O-Group 1

A scheme was developed in the early 1970s and limited attempts to use it for routine typing have given variable results. The scheme has not gained acceptance for epidemiological studies.
CHOLERA ENTEROTOXIN AND ITS RELEVANCE TO PATHOGENESIS, IMMUNITY AND VACCINE DEVELOPMENT

It is now 20 years since experimental evidence first suggested that cholera could be a toxin-mediated disease. Today, it is recognized as the prototype of a number of previously unrecorded diarrhoeal diseases that are also mediated by enterotoxins, some of which are immunologically related to the cholera enterotoxin. Perhaps the most important of these is the Escherichia coli heat-labile toxin (LT). Collectively, the other enterotoxic enteropathies by far exceed cholera as causes of morbidity and mortality in the world. Thus, if appropriate means of inducing antitoxic immunity against cholera can be developed, they might be applicable to prevention of these diseases as well. Similarly, an understanding of the mechanism of action of the cholera enterotoxin at the molecular level may lead to rational methods of pharmacological intervention, and these too may be applicable to the other, similar diarrhoeal diseases.

The cholera enterotoxin (called choleragen in earlier works) has been purified, its structure has been elucidated, and its mode of action has been defined at the molecular level. It is a protein of 84 000 relative molecular mass (RMM) consisting of 2 immunologically distinct regions designated A (active) and B (binding). The B region (previously called choleragenoid) of approximately 56 000 RMM, is composed of non-covalently associated B sub-units of about 11 500 RMM. This region of the toxin is responsible for binding the holotoxin to host-cell membrane receptors that contain a particular glycolipid, the G_m ganglioside. The binding enables the 28 000-RMM A region to penetrate the host cell where it acts, enzymatically, to cleave NAD and to transfer ADP-ribose to the GTP-binding protein associated with the host-cell enzyme, adenylate cyclase. This ADP-ribosylation of GTP-binding protein prevents the breakdown of GTP to GDP, and effectively locks adenylate cyclase in its active state. The net result is the continuous formation of excessive amounts of cyclic-AMP (cAMP). This leads to a rapid sequence of events, as yet unclear, that cause intestine epithelial cells to over-secrete electrolytes followed by water—the cholera stool.

Because of the ubiquity of G_m ganglioside in eukaryotic cell membranes, cholera toxin can activate adenylate cyclase in a variety of cells and tissues with which normally the toxin never comes into contact. The cholera enterotoxin has thus become widely used as an adenylate cyclase/cAMP probe by researchers interested in a variety of cAMP-mediated effects unrelated to cholera. A number of sensitive assays for cholera enterotoxin based on this property have been developed as well. Cultured Chinese hamster ovary (CHO) or Y-1 mouse adrenal tumour cells respond to picogram quantities of enterotoxin with characteristic morphological changes. Cholera toxin also causes a characteristic skin reaction in rabbits and guinea-pigs which serves as a basis for assays. Related enterotoxins are also active in these assays, though exceptions may occur. It is important to recognize that apparently similar activity in organisms of other species or genera may be due to very dissimilar factors, and to exercise caution in the interpretation of the assays.

The cholera enterotoxin is a very effective antigen. In addition to their serological response to the somatic antigens of the cholera vibrios, cholera patients also usually exhibit

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i This section brings up to date the information provided in the Report of the Scientific Working Group on Immunity and Vaccine Development, published as a WHO Memorandum under the title "Intestinal immunity and vaccine development" in Bulletin of the World Health Organization, 57(5): 719-734(1979). For further information, especially on the development of killed bacterial cholera vaccine, the reader is referred to that report.

ADP = adenosine 5'-diphosphate
AMP = adenosine monophosphate
GDP = guanosine 5'-diphosphate
GTP = guanosine 5'-triphosphate
NAD = nicotinamide-adenosine dinucleotide.
antitoxic antibody responses. In many experimental animal studies, antitoxic immunity has been shown to be protective. Antibodies stimulated by the cholera enterotoxin are primarily directed against the B region of the molecule, the immunologically distinct A region being a relatively poor antigen by comparison. It might be predicted that a \( V. \) cholerae O1 strain that produces only the B region of the toxin (an \( A^- B^+ \) mutant), or produces the B with an inactive A region (an \( A^+ B^- \) mutant), could, without causing diarrhoea, effectively deceive the host into producing an immune response similar to that induced by the disease itself, which, as proved in volunteer studies, is highly protective.

A hypotoxigenic mutant developed several years ago was found to be avirulent in human volunteers fed doses of \( 10^{10} \) live vibrios (after sodium bicarbonate). These volunteers were found to be resistant to subsequent challenge with the virulent wild parent \( V. \) cholerae O1 strain. However, further evaluation of this strain as a vaccine was not undertaken, because the resistance was not as solid as that induced by convalescence, and because the mutant: (a) was apparently unstable in that, from one of the volunteers, a colony was isolated that produced larger amounts of cholera toxin; (b) did not colonize very effectively; and (c) could not be expected to induce substantial antitoxic immunity that might extend to the cholera-related enterotoxic enteropathies.

Recently an \( A^- B^+ \) mutant has been isolated from a strain of \( V. \) cholerae O1, El Tor Ogawa. Tests in several laboratories indicate that this mutant colonizes but is avirulent in experimental animal models following extensive serial passages of large inocula. The mutant has induced immunity against challenge with virulent cholera vibrios at 3 weeks in a chinchilla model, although it is not yet clear whether an anti-B antitoxic antibody response is induced. Evaluation of this strain for safety and efficacy as a live vaccine in volunteers is imminent. Another recent mutant (\( A^+ B^- \)) is also ready to be tested in volunteers. If these mutant strains are found to induce effective immunity against cholera, subsequent tests in volunteers will determine whether significant immunity is also produced against related enterotoxic enteropathies, especially LT-producing \( E. \) coli.

Other approaches to effective immunization against cholera could involve the use of non-viable antigens, alone or in combination, administered perorally or parenterally. Volunteer studies suggest that strictly peroral immunization could be effective. A natural toxoid vaccine of only the B region of the toxin has been developed and is currently being evaluated.

Little is known of other virulence factors that might play a role in immunity. The factor or factors responsible for adherence have not been well characterized. Motility of cholera vibrios appears to be an attribute of virulence in some experimental animal models, and it has been postulated that it helps the vibrios to penetrate the mucus layer of the small bowel. The role, if any, of other factors, such as mucinase, neuraminidase, and protease for example, is not understood. A haemolysin has recently been isolated from a strain of \( V. \) cholerae O1, El Tor, which is cytotoxic and lethal in mice. As cholera caused by haemolytic El Tor vibrios is clinically identical to that caused by non-haemolytic El Tor and classical biotypes, this haemolysin probably does not play a role in pathogenesis.

**CHOLERA CONTROL AS AN INTEGRAL PART OF A DIARRHOEAL DISEASES CONTROL PROGRAMME**

Cholera control activities were undertaken in the past by many countries as an *ad hoc* set of actions, initiated only when cholera threatened or struck. A number of countries that have experienced cholera in recent years and are aware of the potential of recent scientific advances are developing programmes for the control of all diarrhoeal diseases. The
essential strategies for such programmes have been outlined elsewhere.\footnote{Unpublished WHO document. Development of a programme for diarrhoeal diseases control (WHO/DDC/78.1).} The Group recommends their adoption in all cholera-infected or cholera-threatened areas, as they offer the best chance of controlling cholera. Where they are operative, patients with cholera are unlikely to die, and panic—which often occurs in newly infected areas following the first deaths—can be prevented.

One of the essential strategies is epidemiological surveillance; if a surveillance programme is functioning properly, outbreaks of cholera and other diarrhoeal diseases can be detected early and investigations and control measures can be initiated before there is any extensive spread. Effective surveillance requires not only the systematic collection of information on the occurrence of cases, but also the analysis and interpretation of data to provide a rational basis for public health action and to enable health officials to determine priorities in allocating limited resources.

The data may be provided by anyone delivering primary health care, such as village health workers, traditional healers, pharmacists, village and religious leaders, teachers and physicians in secondary and tertiary care facilities. Simple reporting forms and a simple case definition should be used. For example, for cholera surveillance in non-endemic areas, the occurrence of an unusually high number of dehydrating diarrhoea cases in persons over 10 years of age is probably enough to suggest that cholera may be present. A report of such cases by providers of primary data should trigger immediate action to strengthen treatment facilities, to confirm the presence of cholera, to determine the vehicle(s) of transmission, and to implement control measures to prevent further spread.

Diagnostic laboratories are important but not absolutely essential. The available laboratory facilities should not be overburdened with a large number of specimens; instead, selectivity in the collection of epidemiologically relevant specimens should be emphasized. In a given point-source epidemic it should not be necessary to collect more than a few specimens for laboratory analysis from patients, together with items of food and water suspected of being involved in transmission.

A well-thought-out plan of action for epidemiological surveillance and epidemic control is essential, as are the personnel and facilities to carry out the plan. The surveillance of diarrhoeal diseases should, wherever possible, be integrated into other national programmes for the surveillance of communicable diseases.

**RECOMMENDATIONS FOR RESEARCH**

In the following recommendations for further research, current knowledge and laboratory methodology are taken into account and the overall objective of the control of cholera and diarrhoea caused by related vibrios is borne in mind. In view of the widely different aspects considered, no attempt is made to establish an overall list of priorities for research. Instead, the recommendations are listed separately for each of the main topics discussed.

**Epidemiology and bacteriology**

**V. cholerae O-Group 1**

(1) The modes of transmission of cholera at the community level should be the subject of more precise studies, coupling the most effective techniques of epidemiology and
environmental microbiology in order to identify the methods that are most likely to be effective in action to control cholera.

(2) Coordinated epidemiological, microbiological, and sociological studies are needed to identify the determinants of cholera endemicity, of the persistence of the disease in the environment, and of its seasonal variations.

(3) Programmes for the continuous surveillance of antibiotic resistance on the part of strains of *V. cholerae* O1 and research into the nature of the resistance factors involved need to be accelerated.

(4) Volunteer studies are needed to define the infective dose of the biotypes of *V. cholerae* O1 more precisely and to determine the influence of food and water on this dose.

(5) The reasons for the low incidence of cholera among infants need to be explained by careful studies in different endemic areas, including several areas where breast-feeding is universal.

(6) Research into the genetic basis for differences between the classical and El Tor biotypes needs to be encouraged with the aim of establishing more stable markers than those used to differentiate these biotypes at present.

*Atypical V. cholerae O-Group 1*

(7) Studies are needed to determine whether there is any association between the failure of the atypical *V. cholerae* O1 to produce toxin *in vitro* and other biochemical reactions.

(8) More volunteer studies should be undertaken to find out whether atypical *V. cholerae* O1 strains that do not produce toxin under laboratory conditions—as measured in a variety of assays—can cause disease in man.

(9) Efforts should be made to test for toxin production in *V. cholerae* O1 isolated from patients, and from the environment in the absence of cases, so as to determine the frequency of atypical *V. cholerae* O1 strains.

(10) The antigenic structure of *V. cholerae* O1 and that of atypical *V. cholerae* O1 should be examined with the aim of preparing a better antiserum for *V. cholerae* O1 for use as an international standard.

*Non-O-Group 1 V. cholerae, Group F vibrios, and related species*

(11) It is important to identify the pathogenic members of these groups and the factors with a bearing on their pathogenicity. The results of studies in animal and other laboratory models to distinguish variations in potential for virulence should ultimately be confirmed in volunteers. The characteristics that can be used for the laboratory identification of the relevant isolates should then be determined.

(12) The pathogenic members of these groups should be looked for in outbreaks and in prospective studies on diarrhoea in order to gather information on their incidence, means of transmission, and clinical features.

(13) A single, internationally accepted serotyping system for non-O1 *V. cholerae* should be developed to facilitate international communication and help achieve a better understanding of the ecology, epidemiology, pathogenicity, and clinical features of this group.

*V. parahaemolyticus*

(14) Studies are needed to elucidate the determinants of virulence in *V. parahaemolyticus* and the relationship of the Kanagawa phenomenon to human enteropathogenicity.

(15) Studies should be undertaken to determine the pathogenesis of *V. parahaemolyticus* gastro-enteritis, particularly as regards the syndrome with the short incubation period.
(16) The mode of transmission in sporadic cases and in outbreaks in which seafood is not involved needs to be clarified, as does the ecology of *V. parahaemolyticus* strains reported to be indigenous to freshwater areas. Further research is also needed to define conditions or means for processing seafood to prevent the multiplication of *V. parahaemolyticus*.  

Environmental surveillance

(17) The ecology of *V. cholerae* O1 in surface waters should be better characterized. If the organisms are truly indigenous to certain habitats, this should be demonstrated. If they are alien to aquatic environments, much more work is needed to identify the factors that influence their ability to survive in these environments.  

(18) Improvements are needed in methods for isolating *V. cholerae* from water and sewage, particularly enrichment techniques, which at present fail to suppress many common aquatic competitors of *V. cholerae*. There is an even greater need for a technique to facilitate the isolation of *V. cholerae* O1 from non-O1 *V. cholerae*. An immunological approach may be feasible, but efforts to identify exploitable physiological differences should also be encouraged.  

(19) Basic studies are needed on the physiological differences between the different types of *V. cholerae* (classical, El Tor, atypical, non-O1) that influence their ability to survive in aquatic environments. These should include survival studies carried out *in situ*.  

Laboratory procedures and phage typing

(20) There is a need for simple, carefully defined methods for determining the pathogenic potential of vibrio species isolated from various sources. International agreement as to the test(s) offering the minimum criteria for establishing pathogenicity should be sought.  

(21) The value of the Maidstone phage-typing system as an epidemiological tool should be assessed in field studies. The isolation of additional phages from various geographical areas may improve the sensitivity of this system and should be encouraged. The feasibility of expanding it to form the basis of an international standard system should also be examined.  

(22) Studies to determine whether phage typing can distinguish atypical *V. cholerae* O1 from *V. cholerae* O1 strains should be expanded and encouraged.  

(23) Simpler and more rapid methods for the laboratory diagnosis of cholera and related infections are still needed. The establishment of a minimum standard of quality for TCBS agar should be encouraged.  

Pathogenesis, immunity, and vaccine development

(24) Further studies are needed to define the factors essential to virulence in *V. cholerae* in the hope that they may lead to additional means of pharmacological intervention. In this regard, research on factors and mechanisms involved in colonization, and in toxin synthesis and transport, could be very productive. The genetic basis of virulence needs to be elucidated, particularly the role of extrachromosomal elements, plasmids, and lysogenic phage in mediating specific virulence factors.  

(25) Immunizing agents (e.g., B-subunit toxoid, an A⁻ B⁺ mutant, and an A⁺ B⁻ mutant) that have recently been developed need to be tested for safety, stability, and ability to elicit substantial protection against both serotypes and biotypes of *V. cholerae* O1 and, if such protection is confirmed, against cholera-related enterotoxic enteropathies.

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(26) Further basic research is needed to provide a more complete understanding of the mechanism of action of cholera enterotoxin, particularly of the rapid sequence of events following activation of adenylate cyclase and resulting in the induction of diarrhoea.

(27) The range of antigenic relationships between enterotoxins from various species of enteropathogens needs to be defined. Additional studies should be directed toward the isolation and characterization of factors related to enterotoxigenicity in organisms other than *V. cholerae* O1.

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