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Original Article

A Six-Year Study on *Vibrio cholerae* in Southeastern Iran

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SUMMARY: Cholera continues to be an important public health problem among many poorer communities. In order to determine the epidemiology of *Vibrio cholerae* in southeastern Iran, 3,594 patients with watery diarrhea, who were referred to the hospitals from Zabol city and 45 neighboring rural populations of Sistan-Blouchestan province, were investigated over a period of 6 years (1997-2002). *V. cholerae* strains were isolated from 362 samples (10.07%). Isolation of *V. cholerae* in this sample decreased from 22.47% in 1997 to 0% in 2002. Individuals of all ages and social and economic strata were affected. Among the patients with cholera in the present study, only 24 (6.6%) lived in an urban area; 270 (74.6%) of the patients had been referred from rural areas, and the remaining 68 (18.8%) were from neighboring Afghanistan. *V. cholerae* O1 Ogawa and NAG vibrios were found in 92.8 and 7.2% of patients, respectively. Among the 362 samples, 244 were collected from inpatients and 118 were from outpatients. Twelve of these patients died because of the severity of their disease, severe dehydration and electrolytes imbalance. The priorities for cholera control remain public health interventions through improved water and sanitation, improved surveillance and access to health care facilities, and further development of appropriate vaccines.

INTRODUCTION

Cholera, an acute intestinal infection caused by the bacterium *Vibrio cholerae*, is a historically feared epidemic diarrheal disease that remains a major public health problem in many parts of Africa, Asia, and Latin America (1,2). In spite of its rarity in developed countries, cholera is still an important infection worldwide (1). In the last decades, attention to cholera epidemiology has increased, as cholera epidemics became a worldwide health problem. Detailed investigation of the interactions of *V. cholerae* with its hosts and with other organisms in the environment suggests that the dynamics of cholera are much more complex than previously thought (3). After the isolation and description of *V. cholerae* by Koch at the end of the 19th century, the 20th century saw the description of the classical and El Tor biotypes and the development of the 7th global pandemic beginning in Sulawesi in 1964, extending through south and southeast Asia and much of Africa in the 1970s, and then crossing into South America in the 1990s (2). In 1992, *V. cholerae* O139, the first non-O1 serotype responsible for epidemic cholera, was isolated in southern India (4). The pandemic continues to grow in many countries (3,5-8). The data on the pandemic indicate that it is still not possible to predict when and where a new epidemic of cholera will start, that appropriate therapy may reduce the mortality to values below 1 %, and that changes in the etiology of this ancient disease are still taking place (1).

V. cholerae is a curved Gram-negative bacillus that belongs to the family *Vibrionaceae* and shares common characteristics with the family *Enterobacteriaceae* (1). The species *V. cholerae* includes both pathogenic and non-pathogenic strains, differing in their virulence gene content, and polysaccharide surface antigens. Only *V. cholerae* O1 and O139 are responsible for the disease defined clinically and epidemiologically as cholera. *V. cholerae* O1 is divided into classical and El Tor biotypes, and into three sero-subtypes, Ogawa, Inaba, and Hikojima. *V. cholerae* O139 has characteristics in common with the El Tor biotype, but differs from O1 in its polysaccharide surface antigen (2).

The survival of *V. cholerae* in aquatic environments, and the potential role of this reservoir in subsequent disease outbreaks have been described in several studies (9). Cholera has unique epidemiologic features. Recognizing the different age groups at risk, depending on the epidemiologic pattern, is useful in designing preventive measures (1). In the present study, we investigated the epidemiology of *V. cholerae* in southeastern Iran.

MATERIALS AND METHODS

A total of 3,594 patients with watery diarrhea were referred to hospitals related to Zabol Medical Sciences College, from Zabol city and 45 neighboring rural populations over a period of 6 years (July 1997-October 2002). This study was performed in Zabol, a large city of Sistan-Blouchestan province, which is situated in southeastern Iran and western Afghanistan.

The diagnosis of cholera remains primarily a clinical diagnosis for the management of individual patients, but laboratory confirmation is necessary in epidemiological investigations and where statutory information is required (2). Therefore, rectal swabs of all of the present subjects were collected and processed according to the standard procedures for isolation of *V. cholerae*. Culturing on thiosulphate-citrate-bile salt-sucrose agar (TCBS) medium, which is the gold standard for cholera diagnosis and confirmation (2), was performed in all of the patients. The stool samples were

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cultured using TCBS. The plates were incubated at 37°C for 18 - 24 h. Suspected non-lactose-fermenting colonies which were oxidase positive and morphologically resembling *V. cholerae* were identified biochemically and serologically using commercially available antisera, i.e., polyvalent O1 *V. cholerae* and monovalent Inaba and Ogawa.

A two-page questionnaire was developed to gather demographic information including name, sex, date of birth, place of birth, clinical status, therapeutic status, result of therapy, *V. cholerae* serotype, and health system status. Based on the questionnaire data, a computerized database was developed using visual basic language programming and access database software. This software allows data entry of all the information recorded by the referring physician on the questionnaire, and also allows direct statistical analysis of data.

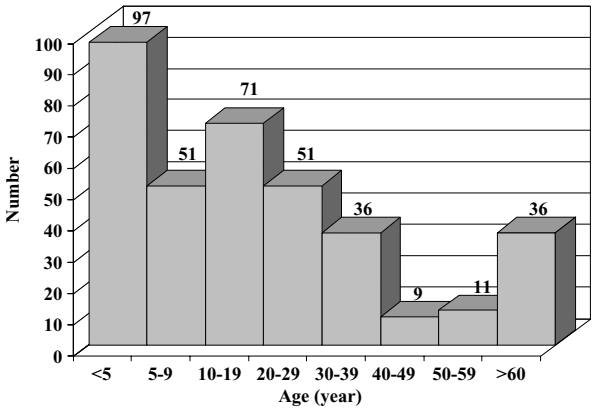

Fig. 1. Age distribution in patients with *Vibrio cholerae*.

RESULTS

V. cholerae strains were isolated from 362 samples (culture detection rate = 10.07%). Over the period of the study, the rate of *V. cholerae* isolation decreased from 22.47% in 1997 to 0% in 2002 (Table 1). Seventeen percent of the affected families had multiple cases, including 39 families with 2 cases, 17 with 3 cases, and 8 with 3 cases.

Individuals of all ages and social and economic strata were affected. Twenty-six percent of all isolates were from children under the age of 5 years. The age range of these 362 patients (218 males and 144 females) was from 1 to 65 years (Fig. 1).

Among the patients with cholera, only 24 lived in an urban area (6.6%); 270 patients (74.6%) with cholera had been referred from a rural area, and the remaining 68 (18.8%) came from neighboring Afghanistan. A total of 222 (61.3%) were among the Iranian population and 140 (38.7%) were among the Afghan population. The drinking-water supply of affected subjects consisted of surface water in 85.1% of cases, including river and spring water in 289 and 19 cases, respectively, and of ground water from wells in 14.9% (54 cases). The distribution of water was accomplished by water pipe (209 subjects), water cask (129 subjects), and water tank (24 subjects). Only 53 (14.7%) of the cases were considered to have a sanitary latrine in their houses, while the home latrines in 272 cases (75.1%) were deemed unsanitary, and 37 cases (10.2%) had no latrine in their homes.

Of the 362 isolates, 336 (92.8%) were found to be *V. cholerae* O1 Ogawa strain. Non-agglutinable (NAG) vibrios accounted for 26 (7.2%) strains. Inaba and Hikojima serosubtypes were not found in our patients.

Among 362 subjects, 244 were inpatients and the remaining 118 were outpatients. Twelve (3.31%) of these patients died because of the severity of their disease, severe dehydration and electrolytes imbalance. Six of the 12 died in the first year of study (1997), and 4 died in 1998. The results of management and therapy in the remaining patients were excellent.

DISCUSSION

Cholera continues to be an important public health problem among many poorer and vulnerable communities, despite the fact that the bacteriology, epidemiology, and public health aspects of the disease were described in detail over a century ago (2). We here investigated 362 cases of cholera, which constituted 10.07% of 3,594 patients with watery diarrhea. This is the first report on the isolation of cholera from this area. Geographic information systems have been utilized to assess the spatial distribution of cholera at the local level, and to demonstrate case clustering and disease risk areas (10). In addition, modelling techniques using climate data, remote monitoring, and geographic information systems may contribute to the prediction of cholera epidemics (11). *V. cholerae* O1 Ogawa were found in 9.35% of 3,594 samples from patients with diarrhea, which was lower than the culture detection rate in previous studies from the neighboring country of Pakistan (6,12). NAG vibrios accounted for 7.2% strains, which was similar to the findings of a previous study from Goa (7).

The hallmark of cholera is the production of watery diarrhea with varying degrees of dehydration ranging from none to severe and life-threatening diarrhea. Laboratory abnormalities reflect the isotonic dehydration characteristic of cholera. The goal of therapy is to restore the fluid losses caused by diarrhea and vomitting (1). Such management was

Table 1. Patients with *Vibrio cholerae* from 1997 to 2002

Year	No. of patients with diarrhea	*V. cholerae* *n* (%)	Sex		Treatment		Referral center			Serotype	
			Male	Female	Inpatient	Outpatient	Urban area	Rural area	Afghanistan	O1 Ogawa	NAG
1997	712	160 (22.47)	99	61	125	35	6	123	31	148	12
1998	1,413	119 (8.42)	65	54	65	54	11	89	19	119	0
1999	739	68 (9.20)	45	23	52	16	5	55	8	54	14
2000	314	15 (4.78)	9	6	2	13	2	3	10	15	0
2001	281	0	0	0	0	0	0	0	0	0	0
2002	135	0	0	0	0	0	0	0	0	0	0
Total	3,594	362 (10.07)	218	144	244	118	24	270	68	336	26

performed for all of our patients, 67.4% of whom were admitted to a hospital. However, 3.31% of the patients died because of the severity of their disease, severe dehydration and electrolytes imbalance.

In our study, cases of cholera were identified each year, both in adults and children, each year from 1997 to 2000. Despite the rapid urbanization and increasing affluence in southeastern Iran, cases of cholera had remained frequent right up until the end of the previous century, since then, the number of patients with cholera has decreased drastically, from 160 (22.47%) in 1997 to 0 in 2002 in the present cohort. There are many factors involved in this success. However, the most imortant seems to have been improving the infrastructure of the water supply system and taking actions to train and strengthen local capabilities to foster greater acceptance and commitment from the community towards their own water supply system. The reproduction rate of cholera in a community is defined as a product of social and environmental factors. The importance of the aquatic reservoir depends on the sanitary conditions of the community (3). There are several means by which we tried to prevent waterborne disease transmission. Among these are two categories of infection-control practices. The first category represents establishment of physical barriers to prevent contamination of water used for recreation as the source of drinking water. The second category consists of water disinfection treatment. Transmission of cholera was probably significantly reduced when uncontaminated water was provided to our population in the year 2000. Providing potable water and ensuring proper management of excreta to avoid contamination of other water sources were important measures to reduce cholera transmission.

Education of the population at risk on appropriate hygienic practices is always recommended. Identification of local customs that place people at risk is eliminating unhygienic practice (1). Also, early detection of cases and an effective surveillance system to direct scarce resources will result in more timely and appropriate case management. Further development of cholera control will require a better understanding of *V. cholerae* ecology and epidemiology.

Priorities for cholera control remain public health interventions through improved water and sanitation, improved surveillance and access to health care facilities, and further developments in appropriate vaccines. A greater understanding of the pathogen, its biology, ecology, and epidemiology, and strategies for treatment and prevention are essential to guide policies and programs for the control of cholera. The global reduction of the burden of cholera as a public health problem requires international and national action to improve public health and reduce the vulnerability of disadvantaged communities (2).

It is important to consider *V. cholerae* when empirically treating diarrheal diseases in our region. Adequate measures to improve hygiene and sanitation and supply of safe potable water are needed to prevent any future epidemic of cholera in Zobol city. As with sanitation, water supply must be seen as a complex system of interrelated factors. The successful water supply system is therefore to be found in the successful organization of the factors affecting the health and social organization of the community. The provision of an adequate supply of potable water should be seen as just one part of this system, since the improvement of water quality alone would have little effect on water handling practices and the subsequent contamination of other stored water. Domestic activities related to the storage and use of water may have a more important bearing on fecal-oral disease transmission. The mere material improvement of water supplies would doubtless prove to be less effective than health education on the causes and means of avoiding disease. Primary health care education is thus a vital component in the prevention of cholera.

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