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Cholera in a developing megacity; Karachi, Pakistan

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SUMMARY

Despite rapid urbanization and increasing affluence in Karachi, cases of cholera are frequent. We analysed computerized isolation data from the AKUH Clinical Microbiology Laboratory, Karachi, from 1990–6 to examine microbiological, temporal and demographic trends in *Vibrio cholerae* infections. During this period 888 strains of *V. cholerae* (566 *V. cholerae* serogroup O1, and 204 *V. cholerae* serogroup O139) were isolated from specimens from 886 patients; 214/464 were adult inpatients, and 250/464 paediatric inpatients, the remaining 422 outpatients. Isolations peaked between June and August. Overlapping epidemics occurred in 1993 and 1994 of serogroup O1 (May to August), and serogroup O139 (August to October). All ages and social and economic strata were affected. Forty-four percent of all isolates were from children under the age of 5 years. The mean age of all patients with serogroup O1 infections was 19·6 years ($\pm$0·9) compared with 36·7 ($\pm$1·7) for serogroup O139 infections ($P < 0{\cdot}0001$, *t* test). More than a quarter (27%) of all serogroup O1 isolates were from babies under 2 years of age. One patient had a serogroup O1 infection followed by a serogroup O139 infection 1 year later. Another patient was infected with serogroup O1 strains 5 years apart. Emergence of resistant strains was observed, but by 1996 serogroup O139 had disappeared. An aquatic organism, cholera nevertheless continues to take its toll in this city of 11 million situated in a desert.

INTRODUCTION

Cholera has an ancient reputation as a killer disease [1]. It is said to have originated in Asia, where it has a long history of major epidemics. Periodically, emergence of new strains of the causative organism *V. cholerae* has been a mechanism by which the bacterium has been able to reestablish itself in partially immune populations and then disseminate across the globe. In this way since the early 19th century, cholera has swept through the world in seven great pandemics [2]. The ongoing seventh pandemic is caused by *V. cholerae*, serogroup O1, El Tor strain. This strain emerged first in Hong Kong in 1961, and clonal variants spread virtually worldwide replacing the classical strain of *V. cholerae* and one clone finally reaching Latin America in 1991 [3, 4]. In late 1992 a new mutant of the original *V. cholerae* El Tor serogroup O1 strain emerged [5]. It was designated *V. cholerae*, serogroup O139, and it bears a mutation in the chromosome of the bacterium, altering its antigenic profile [6]. It was first seen in Pakistan in 1993 [7, 8]. At the same time resistance to cheap and non-toxic antimicrobials was observed [9]. Cholera cases appeared to be on the increase.

We sought to describe the circulation and emergence of cholera strains in Karachi, a rapidly developing megacity of South Asia with a population estimated at about 11 million. Despite a robust economy, a rapidly expanding population combined

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with civil unrest and a crumbling infrastructure deny basic sanitation and clean water to many of its inhabitants. We accessed computerized laboratory reports from June 1990 to August 1996, and extracted data on isolates of *V. cholerae* over that period in a busy hospital microbiology laboratory serving both inpatients in a tertiary care facility and outpatients from all over the city.

MATERIALS AND METHODS

Patients and specimens

Patients were either inpatients at the Aga Khan University Hospital (AKUH), Karachi, attended the emergency room for diarrhoeal disease at the same hospital, or were patients who submitted stool samples for culture directly to the AKUH laboratory or one of its collection points throughout Karachi. All specimens were processed at the Clinical Microbiology Laboratory at the AKUH.

Laboratory techniques

Between June 1990 and January 1993, stool specimens were only processed for *V. cholerae* if specifically requested on the request form. Beginning January 1993, however, all stool specimens with a loose watery appearance were routinely cultured for *V. cholerae*. Swabs were dipped into the stool specimens and then used to plate directly onto thiosulfate-citrate-bile salts-sucrose (TCBS) and MacConkey agar and incubated at 37 °C overnight [9]. Yellow colonies on TCBS agar were subcultured further onto TCBS, MacConkey and nutrient agar. Definitive identification of pure culture of *V. cholerae* was performed using API20E (analytical profile index 20, enterobacteriaceae, Bio Merieux France, supplemented with tube sugars (sucrose, lactose, inositol), indole, motility and oxidase tests). *V. cholerae* serogroup and subtypes were determined from nutrient agar subculture, using slide agglutination techniques with polyvalent antisera for Ogawa and Inaba strains of *V. cholerae* serogroup O1-(Murex Diagnostics Ltd. England), and for serogroup O139 (Deinka Seiken Co. Ltd, Tokyo, Japan). To increase the speed and accuracy of isolation and reduce cost compared with the conventional method, routine isolation from September 1994 was performed using tellurite taurocholate gelatin agar (TTGA) [10–12]. Final identification of overnight colonies with a grey zone of gelatin hydrolysis was by direct agglutination from the TTGA plates, as described. Antimicrobial susceptibility was determined by disk diffusion techniques (Kirby–Bauer) using furazolidine 15 μg, ampicillin 25 μg, chloramphenicol 30 μg, erythromycin 15 μg, cotrimoxazole 25 μg, ofloxacin 10 μg, tetracycline 30 μg, nalidixic acid 30 μg.

Data management and analysis

All data were directly entered into a computerized reporting system. Data were downloaded, managed in Foxprow and analysed using SAS and EpiInfo 6.0.

RESULTS

Between June 1990 and 31 August 1996, 888 strains of *V. cholerae* were isolated from 886 patients, 389 (44%) females and 497 (56%) males (2 sex not stated). 52% (464/888) of isolates were from inpatients. 214 (46%) of the 464 patients were admitted to the adult wards of the AKUH. 250 of the inpatients (54%) were admitted to the paediatric wards of the same hospital (birth to 14 years of age). 43 (9%) of the 464 inpatients occupied private rooms at the time of stool collection. 278 (31%) further patients presented to the emergency room (162 patients) or outpatient clinics, and 116 specimens (13%) were brought directly to the laboratory by patients or relatives ('direct referrals'), some but not all with a form or letter of request from a local practitioner. Twenty-one further specimens came from laboratory collection points in other parts of Karachi, and 125 did not have a designated location recorded at the time of specimen collection, however, most of these were likely to have also to have been 'direct referrals'.

Seasonal and annual trends are illustrated in Fig. 1. Each year, *V. cholerae* isolates first appeared between April and June, peaking in July and August. Between October and March few if any *V. cholerae* were isolated. 1993 and 1994 were epidemic years for cholera compared with 1995 and 1996. 1994 was a year of abnormally high rainfall in July and August, and rains were also plentiful in July of 1995. There was no measurable rainfall at all during the whole of 1993.

684 of the 888 isolates were identified as *V. cholerae* E1 Tor, serogroup O1, all of which were subgroup Ogawa, with the exception of four subgroup Inaba strains. *V. cholerae* serogroup O139 was first identified

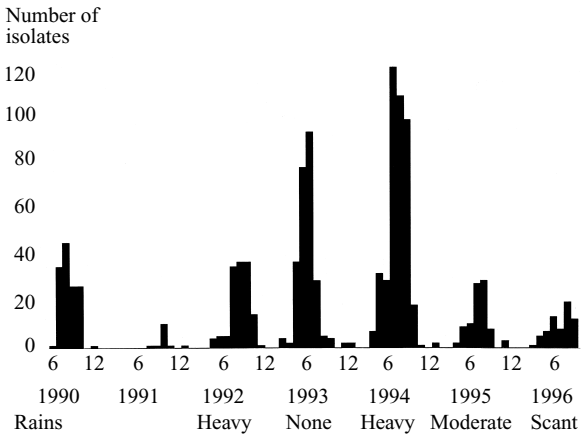

Fig. 1. Epidemic curve of cholera isolates from 1990–6 showing seasonal and annual fluctuations. (Isolation in 1991 was only performed on request, and data for 1991 should therefore be disregarded.)

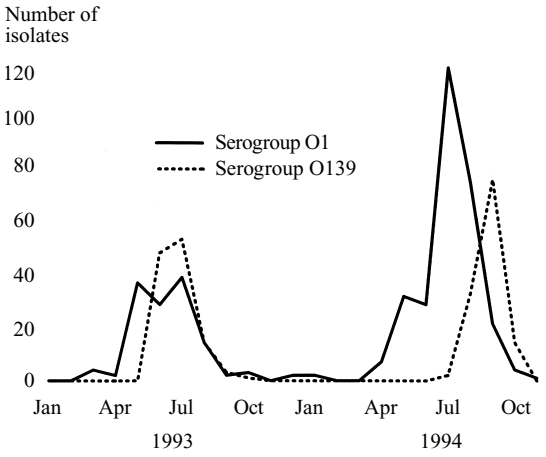

Fig. 2. Overlapping epidemics of the two strains of *V. cholerae* in 1993 and 1994.

in July 1993, and since then 204 isolates of this strain have been made; 88 of 189 (47%) *V. cholerae* isolates in 1993, 113 of 338 (33%) in 1994, and 3 of 68 (4%) in 1995. No strains of *V. cholerae* serogroup O139 were isolated in 1996. Fig. 2 shows the overlapping temporal patterns of the two epidemic strains in 1993 and 1994. Serogroup O139 strains did not appear until July and August each year, and continued to circulate into September and October. In August 1994, the apparent replacement was very rapid, occurring over a period of two weeks.

Over the 5-year period, 44% (386/888) of all isolates were from children under the age of 5 years. Only 12% of isolates were from children and young adults between the ages of 5 and 20 years, and a further 12% from persons over the age of 60. The

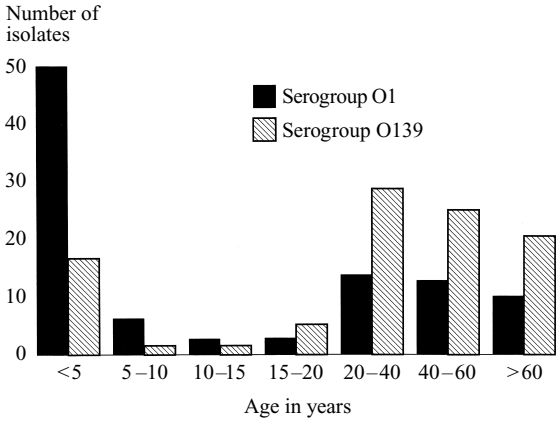

Fig. 3. Age distribution of patients infected with *V. cholerae* serogroups O1 (1990–6) and O139 (1993–5).

highest single group of isolates were from children under the age of 2 years from whom we obtained 27% (242/684) of all serogroup O1 isolates. The mean age of all patients with serogroup O1 infections was 19·6 years ($\pm$0·9) compared with 36·7 years ($\pm$1·7) for serogroup O139 infections ($P < 0{\cdot}0001$, *t* test). This distribution by age of patients infected with the different strains is shown in Fig. 3.

Two patients had two separate episodes of culture-confirmed cholera over the 6-year period. One was a woman aged 25 years from whom *V. cholerae* serogroup O1 was isolated in 1990 and again in 1995. The other was a 65-year-old female from whom *V. cholerae* serogroup O1 was isolated in 1992, and *V. cholerae* serogroup O139 in 1993.

We also reviewed the reported antimicrobial sensitivities of the oranisms over the 5-year period. *V. cholerae* serogroup O1 was sensitive to a number of antimicrobials at the beginning of the period. Between 1991 and 1993 most serogroup O1 strains became resistant (Table 1). *V. cholerae* serogroup O1 strains have remained sensitive to nalidixic acid and ofloxacin throughout the period of observation. *V. cholerae* serogroup O139 isolates show uniform sensitivity to tetracycline.

DISCUSSION

Cholera, a preventable but life-threatening disease, remains a major public health problem in Pakistan, despite the absence of notifications by that country to WHO. In Asia the disease is expanding. WHO has recorded a 17% increase in cholera cases in 1994 compared with 1993, and the appearance of a new

Table 1. *Percentages of isolates sensitive to commonly used antimicrobials by year**

	1990	1991	1992	1993	1994	1995	1996
V. cholerae O1							
Tetracycline	109/109† (100)‡	7/7 (100)	85/86 (99)	11/64 (17)	39/220 (17)	3/65 (7)	5/54 (9)
Cotrimoxazole	2/113 (2)	1/9 (11)	41/112 (37)	4/98 (4)	2/218 (1)	0/64 (0)	2/51 (4)
Erythromycin	107/118 (90)	1/1 (100)	ND§	11/65 (17)	91/224 (41)	7/65 (11)	24/54 (44)
Chloramphenicol	73/97 (75)	8/9 (89)	97/106 (92)	22/24 (92)	13/19 (68)	40/64 (63)	37/52 (71)
Nalidixic Acid	101/102 (100)	9/9 (100)	40/40 (100)	94/94 (100)	221/224 (98·7)	65/65 (100)	53/54 (98)
Ofloxacin	113/113 (100)	8/8 (100)	111/111 (100)	99/99 (100)	216/216 (100)	63/63 (100)	54/54 (100)
V. cholerae O139							
Tetracycline	—	—	—	85/87 (99)	111/112 (99)	3/3 (100)	
Cotrimoxazole	—	—	—	3/88 (3)	1/83 (1)	0/3 (0)	
Erythromycin	—	—	—	8/85 (9)	28/112 (25)	1/3 (33)	
Chloramphenicol	—	—	—		(65)	(100)	
Nalidixic acid	—	—	—	84/85 (99)	89/90 (99)	3/3 (100)	
Ofloxacin	—	—	—	87/87 (100)	89/89 (100)	3/3 (100)	

* Since the number of organisms tested each year often varied considerably we have shown actual numbers as well as percentages. These variations were due to a number of factors such as temporary changes in laboratory testing policy, unavailability of discs, and other minor problems.

† Number sensitive/number tested (percentage sensitive).

‡ Numbers in parentheses indicate percentages.

§ Not done.

strain affecting ten Asian countries by the end of 1994 [13]. Karachi is a developing Asian megacity with a vibrant economy, but with significant social disruption. The population is estimated at around 11 million, for most of whom there is inadequate sewage and clean water supply. In such conditions, enteric infections continue to take their toll.

Though our data reflect only patients from whom specimens were sent for routine diagnosis, and lack denominators, they confirm the continuing presence of cholera in Karachi, and illustrate its seasonal and temporal periodicity. Cholera cases peak each year between May and August with epidemic and non-epidemic years. Karachi is situated in a desert and rainfall is sporadic, usually only between June and August. In years such as 1994, the city was flooded, with widespread overflowing sewers; other years, such as 1993 there was virtually no rain at all. Cholera appears each year before the rains, and epidemic years appear at least from our data to have occurred independent of rainfall.

In 1993 the new strain of *V. cholerae*, serogroup O139, established itself in Karachi [7]. During 1993 and 1994 Karachi experienced overlapping, but distinct epidemics of both strains. We observed that, serogroup O139 never wholly replaced serogroup O1, and by 1996 it had disappeared. It is still unclear whether this disappearance is permanent, or whether this strain will re-emerge in subsequent epidemic years. This strain has shown diminished ability to maintain its epidemic potential in Bangladesh, and it has been suggested that one reason for this in Bangladesh may be that it is less able to persist long-term in the aquatic environment [10, 14]. Karachi, is in a semi-desert area, and the strain may not be able to maintain itself there outside the human host.

Like most enteric diseases in an endemic setting, cholera in Karachi is a disease of young children. In

contrast to reports from Bangladesh, we find that children under two are the group from whom we obtain the most isolates [10]. In 1993 and 1994, *V. cholerae* serogroup O139 because of its altered antigenic profile was able to infect older people who presumably had complete or partial immunity to the serogroup O1 strain of *V. cholerae*. One of our patients illustrated this point by experiencing two episodes of cholera in 2 consecutive years caused by the 2 different strains. Another patient was infected by a *V. cholerae* serogroup O1 strain on 2 occasions with an interval of 5 years, illustrating that cholera may not induce lasting immunity even to the original infecting strain. In 1993, the year of the first reported appearance of serogroup O139 in Karachi, we saw the age distribution of diarrhoeal patients shift significantly towards the adult age group (Fig. 3). This age difference did not persist in 1994, despite continued circulation of *V. cholerae* serogroup O139. It is possible that the epidemic in 1993 may have induced immunity in many city residents, in which case there must have been a large number of asymptomatic infections in 1993 and the illness-to-infection ratio of serogroup O139 may be lower than that for serogroup O1.

Our data contrast markedly with those from Thailand where cholera is endemic at relatively low levels, and most cases are among refugee populations and in coastal areas, not in the city of Bangkok [15]. Presumably the low level of infection in the cities and in children reflects economic advancement and improved living conditions. The mean age of patients with acute cholera in Karachi more closely resembles that in the rest of the Indian subcontinent where social conditions are more comparable [10]. Despite this association with poverty, 9% of the patients in our study were admitted to expensive private rooms in the AKUH. Thus in a city with sanitary infrastructure like that of Karachi, personal wealth affords no protection. Equally visitors and tourists continue to be at risk [16, 17].

Strain variation was also observed in the changing antibiograms of isolates over the period of observation, with increasing resistance to commonly used antimicrobials over the 5-year period. Resistant strains have been reported from Russia, India, Africa and South America [18–21]. In 1982 in Thailand a multiply-resistant strain with a group C plasmid encoding resistance to seven antibiotics was reported to have caused an epidemic in a single pediatric ward in 1982 [22]. In Tanzania and in South America resistance to tetracycline was demonstrated to have developed very rapidly following use of that drug as prophylaxis in contacts [20, 21]. In Karachi widespread over the counter availability of antimicrobials undoubtedly leads to resistance as was also thought to be the case in Ecuador [20].

These data clearly illustrate that cholera remains a major public health problem in Karachi particularly for the oldest and the youngest citizens, threatening rich and poor alike.

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