

# Re-emergence of *Vibrio Cholerae* O139 in Pakistan: report from a Tertiary Care Hospital

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## Abstract

### Objective:

This study reports re-emergence of *Vibrio cholerae* O139 in Pakistan in 2000-2001 from a tertiary care hospital in Karachi, Pakistan.

### Methods:

This descriptive study was conducted from 2000-2001. Stool samples were taken from inpatients or those referred to the laboratory from other hospitals, clinics and general practitioners. Samples were processed and *Vibrio cholerae* isolates were identified according to standard protocols. Tellurite Taurocholate Gelatin agar was used as a selective medium for *Vibrio cholerae*. Serogroups were identified by slide agglutination with polyvalent antisera. Antimicrobial sensitivities were performed by Kirby Bauer technique. Data was entered and analyzed using SPSS, p values were calculated using t test and two independent samples test.

### Results:

During the study period, 144 samples were found to be infected with *Vibrio cholerae* O139 in comparison with 545 *Vibrio cholerae* O1. Infection with O139 was characteristically observed in the older population (mean age = 40 years) in contrast with *Vibrio cholerae* O1 strains (mean age = 23 years) (p. value= <0.001). Sensitivity pattern of 2000-2001 *Vibrio cholerae* isolates was markedly different to that of 1993-1994. The earlier isolates were resistant to Cotrimoxazole (99%) and Chloramphenicol (35%) whereas the recent isolates are almost 100% sensitive.

### Conclusion:

In conclusion this re-emergent strain seen 6 years after previous episode infected large number of people especially older population suggesting that prior infection with O1 does not provide immunity against O139 and therefore *Vibrio cholerae* O139 has a potential to cause a major epidemic in an immunologically naïve population (JPMA 53:335;2003).

## Introduction

*Vibrio cholerae* non-O1 sero-groups were not recognized as cause of epidemics of diarrhea until 1992, when an epidemic of cholera like disease due to *Vibrio cholerae* O139 broke out in southern Madras.<sup>1</sup> Over the next few months this epidemic spread to other parts of India and Bangladesh<sup>2-5</sup> reaching Pakistan in 1993 where it began to cause disease along with *Vibrio cholerae* Ogawa, (the predominant strain at that time).<sup>6</sup> However, *Vibrio cholerae* O139 never completely replaced *Vibrio cholerae* O1 and by 1996, O139 strain totally disappeared from the country<sup>7</sup>, although periodic resurgences of *Vibrio cholerae* O139 were reported from other parts of world.<sup>8-11</sup> More recently since July 2000 however we have noted a recurrence of *Vibrio cholerae* O139 amongst

our laboratory isolates and we are now reporting this resurgence over the years 2000 and 2001.

### **Materials and Methods**

The study was conducted at a 550-bed tertiary care hospital located in Karachi, Pakistan. The clinical microbiology laboratory receives samples of patients presenting to hospital itself as well as outside referrals from other hospitals, clinics and general practitioners across the city. Stool samples were from patients seen at the hospital, or those referred to the laboratory from other hospitals, clinics and general practitioners across the city. All stool samples for culture were plated directly on MacConkey, Salmonella-Shigella, Campylobacter and Tellurite Taurocholate Gelatin agar (Oxoid). Stools were also inoculated in Selenite F broth and Alkaline Peptone Water and incubated overnight at 37°C. Suspected colonies from TTGA were confirmed by using API 20 E (Analytical profile index 20, enterobacteriaceae Bio Merieux France). Serogroups were identified by slide agglutination with polyvalent antisera for Ogawa and Inaba strains (Murex Diagnostic Limited), and for serogroup O139 (Dienka Sieken Co. Limited Japan). Antimicrobial sensitivities were performed by Kirby Bauer disc sensitivity technique.

### **Statistical Analysis:**

Data was entered and analyzed using SPSS, p values were calculated using t test and two independent samples test.

### **Results**

During the year 2000-2001, a total number of 8987 stool samples were processed for culture at Aga Khan University Hospital laboratory. Of these 689 (7.6%) yielded growth of *Vibrio cholerae*. The total numbers of O139 strains isolated during this period were 144 (21%) as compared to 545 (79%) *Vibrio cholerae* O1. The frequency of cholera varies from year to year; more isolates were noted in 1993-94 as compared to 1995-96. The disease frequency increased again in 1997 and this trend shows no sign of abating in recent years (Figure 1).

In 2000, cholera isolates were first noted in June and the O1 strains peaked in the July although the highest numbers of O139 isolates were seen in October. Whereas in 2001 cholera started to emerge in May and both O1 and O139 strains peaked in July-August. This trend was similar to that observed in 1993-94 when epidemic of cholera again peaked in summer months. It was noted moreover that O139 never completely replaced O1 and O1 strain remains the main etiologic agent of cholera in our setting. (Figure 2). It was interesting to note that the patient population affected by the different strains differs in its age distribution; with *Vibrio cholerae* O139 tending to affect the older population (mean age 40 years versus 23 years for *Vibrio cholerae* O1 (p value <0.001)) (Figure 3).

Although cholera is characteristically associated with watery diarrhea, microscopy showed that 104 out of 144 (72.2%) stool samples with *Vibrio cholerae* contained significant (>8/hpf) numbers of fecal leukocytes. Thus indicating association of inflammatory process along with toxin mediated illness.

Antibiotic sensitivity pattern of *Vibrio cholerae* O1 and O139 is shown in table. In comparison with the previous epidemic of *Vibrio cholerae* O139 in 1993-94 the sensitivity pattern of this resurgent strain has changed with increased sensitivity for

Cotrimoxazole and Chloramphenicol in the more recent isolates. This change in sensitivity pattern also held true for *Vibrio cholerae* O1.

### **Discussion**

Cholera remains a major health problem in Pakistan responsible for a large number of cases each year. The disease peaks each year between May and August usually associated with the monsoon rainfall in the presence of poor sewerage system and inadequate water supplies.

In both time periods 1993-94 and 2000-2001, O1 cholera remained the main etiologic agent of cholera in the community despite resurgence of O139 strain.<sup>7</sup> A possible explanation for this phenomenon could be that the transmission of O139 is not as efficient as that of O1 strain due to perhaps to comparatively lower survival of O139 in the environment.

The current epidemic of O139 is noted 6 years after the 1993-1994 epidemic in Pakistan. There are similar reports of resurgence from India and Bangladesh in 1998, where it was analyzed that the re-emergent strain was a new ribotype with altered rRNA distinctly different from the previous strains. Re-emergent strains collected from India in 1998 moreover showed altered sensitivity pattern as compared with earlier O139 isolates. It is thought that the change in sensitivity was due to considerable reassortment in genetic element encoding antimicrobial resistance especially a deletion of a region in the SXT element.<sup>12-16</sup> While ribotyping was not carried out on our isolates, the dramatic shift in sensitivity patterns could be explained by similar reassortment of genetic elements.

It was also noted that O139 strain involves a comparatively older population. Our finding is consistent with previous studies of O139 strains in Pakistan reported by Sheikh et al<sup>7</sup> and Lalani et al (unpublished data) where O139 was again reported to affect an older age group. The higher prevalence in the older age group suggests that prior infection with O1 does not provide immunity against O139. It is likely that the antigenic profile of *Vibrio cholerae* O139 is different from that of *Vibrio cholerae* O1 and that *Vibrio cholerae* O139 therefore has the potential to cause a major epidemic in an immunologically naïve population.

Although cholera is classically associated with non-inflammatory diarrhoea, presence of fecal leucocytes and red blood cells in stool of cholera patients have been reported.<sup>17</sup> Similar finding in stool samples from our patients supports earlier data and also implies involvement of inflammatory mechanisms in the pathogenesis of *Vibrio cholerae* O139 infection.

Majority of the patients in our study were refugees and immigrants from Afghanistan living in crowded refugee camps under compromised living conditions, emphasizing the role of poor hygiene and over crowding in the transmission of cholera. Moreover immunity in these patients was likely to have been suppressed due to inadequate nutrition and various infections, thus increasing their susceptibility to this resurgent infection.

In conclusion this re-emergent strain, seen 6 years after previous episode, infected large number of people especially older population. This suggested that prior infection with O1 does not provide immunity against O139 and therefore *Vibrio cholerae* O139 has a potential to cause a major epidemic in an immunologically naïve population.

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