

VIBRIO CHOLERAЕ; WATERBORNE OUTBREAK

DR. AMIR MOHAMMAD BABAR

MBBS, D.C.H.M. Phil(Pb)

Associate Professor Microbiology
Bolan Medical College, Quetta.

DR. CHANDI KAPOOR

MBBS. M.Phil

Associate Professor Pathology
Bolan Medical College, Quetta.

DR. MOHAMMAD IQBAL

MBBS, M. Phil

Assistant Professor Pathology,
Pathology Department
Bolan Medical Complex Hospital, Quetta.

Dr. Mohammad Hanif Mengal

MBBS, M. Phil

Assistant Professor Pathology
Bolan Medical College, Quetta.

Dr. Ghulam Sarwar Pirkani

MBBS, M. Phil

Professor of Microbiology,
Department of Pathology
Principal Research Officer PMRC,
Bolan Medical College, Quetta.

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ABSTRACT... **Objective:** To isolate the etiological agent of diarrheal outbreak, identification, antibiogram of isolated bacteria. **Patients and Methods:** Stool samples from patients and water samples from reservoir of water collected in transport media. Culture and sensitivity test were performed in Microbiology Laboratory of Bolan Medical Complex Hospital Quetta. The isolated strain was confirmed by National Institute of Health Laboratory Islamabad and Agha Khan University hospital laboratory Karachi. The outbreak was controlled by preventive measures. **Results:** More than five thousand people affected in the outbreak, including all age and both sexes. Twenty three deaths (0.4%) occurred in this outbreak. The stool samples from patients and from 5 water reservoir tanks collected for analysis of etiological agent responsible for the outbreak. The stool and water samples revealed isolation of *Vibrio cholerae* Inaba, El Tor, which was sensitive to Ampicillin, Ciprofloxacin, Tetracycline, Doxycycline and intermediate to Chloramphenicol and Erythromycin. Resistant to Nalidixic Acid Polymaxin B, and Co-trimoxazole. **Conclusion:** The *Vibrio cholerae* Inaba, El Tor was the causative agent of this outbreak and the first outbreak which occurred in Balochistan due to *Vibrio cholerae* Inaba, El Tor.

INTRODUCTION

Vibrio cholerae is a known etiological agent for large outbreaks of severe diarrheal disease. Many pandemics have been reported in history. The first long-distance spread of cholera to Europe and the Americas began in 1817 and by the early 20th century, six waves of cholera had spread across the world in devastating epidemic fashion^{1,2}. In 1961, the "El Tor" biotype re emerged to produce a major epidemic in the Philippines and to initiate a seventh global pandemic. Since then this biotype has spread across Asia, the Middle East, Africa, and more recently, parts of Europe^{3,4}.

WHO report 2000 states that in 1998, diarrhea was estimated to have killed 2.2 million people, most of whom were under 5 years of age). Each year there are approximately 4 billion cases of diarrhea worldwide.

Cholera and dysentery cause severe, sometimes life threatening forms of diarrhoea *Vibrio cholerae* is a

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Correspondence Address:
Dr. Ghulam Sarwar Pirkani
Flat No B-3, BMC Complex, Quetta.
gspirkani@yahoo.com

known etiological agent for large outbreaks of severe diarrheal disease. (WHO, 2000)

In December, 1992, a large epidemic of cholera began in Bangladesh, and large numbers of people have been involved. The organism has been characterized as *Vibrio cholerae* O139 "Bengal". It is derived genetically from the El Tor pandemic strain but it has changed its antigenic structure such that there is no existing immunity and all ages, even in endemic areas, are susceptible⁵. The epidemic has continued to spread, and *V. cholerae* O139 has affected at least 11 countries in southern Asia. Specific totals for numbers of *V. cholerae* O139 cases are unknown because affected countries do not report infections caused by O1 and O139 separately^{6,7}. In Pakistan outbreaks due to *Vibrio cholerae* O139 have been reported from Karachi 1993⁸ and from Sibi city of Balochistan in 1994⁹.

Vibrio cholera O1 strain outbreak reported from Rawalpindi¹⁰ and Mansehra, Sawat and Muzafarabad^{11,12} while an outbreak of severe diarrheal disease occurred in Drakala Village of Wadh area in Khuzdar district of Balochistan province, in July 2004 due to the infection of *Vibrio cholerae* Ogawa, El Tor strain, and the source was the drinking water¹³.

In July 2005 a severe outbreak of diarrheal disease occurred in Zhob City, the headquarter of district Zhob in Balochistan. It affected the almost all areas of the city. We have collected stool samples from patients and water samples from water reservoirs for isolation of etiological agent and its determined its antibiogram.

PATIENTS AND METHODS

Collection of specimen and processing

This study was conducted in Bolan Medical College Hospital Quetta. In July 2005, an outbreak of severe diarrheal disease occurred in Zhob city the headquarter of district, the northern region of Balochistan province, in month of July 2005. Most of the population of the city suffered from the disease and Twenty three patients expired. five samples of stool from the patients and five samples of water from the reservoir tanks of water, were collected. The samples were inoculated in Cary- Blair

transport media. On reaching to Quetta samples were inoculated on TCBS agar, Mac Conkey agar, Blood agar and XLD agar. The plates were incubated at 37 C for 24 hours. Next day the isolates were examined by gram staining, motility, colonial characterization and biochemical profile by standard techniques¹⁴. The serological identification and typing was carried out by *V. cholerae* anti sera (Denka Seiken, Japan). The strain and antibiogram was further confirmed by National institute of Health Islamabad and Aga Khan University Hospital Karachi Laboratory.

Detection of Antimicrobial Susceptibility

The antimicrobial susceptibility was determined by disk diffusion test following Kirby Bauer method using Mueller Hinton agar. The standard antimicrobial disks (Oxoid, BBL) ampicillin 10ug (AMP), chloramphenicol 30ug (CAP), co- trimaxazole 23.75 ug (SMX), nalidixic acid 50ug (NA), tetracycline 30ug (TE), polymaxin B 100 i.u. unites (PB) doxycycline (30 ug DOX) and ciprofloxacin 5 ug (CIP), were used.

RESULTS

Five thousand four hundred and thirty three persons affected including all age and both sexes. Twenty three deaths (0.4%) reported. In this outbreak 15 female and 8 male out of which 12 were adults and 13 children died, which occurred in month of July 2005 The stool samples were collected from 5 patients and 5 water reservoir tanks including the main source of water "Saleza Village" for analysis. All samples were inoculated on TCBS agar, MacConkey agar, Blood agar and XLD agar. The plates were inoculated at 37°C. After 24 hours incubation the isolated bacteria were examined by gram staining, colonial characterization, and biochemical tests by standard techniques. The serological typing was done by *Vibrio cholerae* anti sera supplied by Denka Seiken, Japan. After The stool and water samples revealed isolation of *Vibrio cholerae* Inaba, El Tor. We also confirmed our isolate form Agha Khan University hospital laboratory Karachi and National Institute of Health Islamabad. They were sensitive to Ampicillin, Ciprofloxacin, Tetracycline, Doxycycline and intermediate to Chloramphenicol and Erythromycin. Resistant to Nalidixic Acid Polymaxin B, and Co-trimaxazole.

DISCUSSION

Every year outbreaks of severe gastroenteritis occur in different parts of Balochistan province specially in summer season in southern region where climate is very hot. *Vibrio cholera* 0139 outbreak was reported from Sibi city of Balochistan in August 1994⁹. The *Vibrio cholerae* Ogawa has been isolated from Khuzdar in July¹³. This outbreak was also in month of July.

Human beings are natural reservoir for *Vibrio cholerae*, and they pass the organism in stool contaminating the water or food sources. In this outbreak there was rain in the area two days before the outbreak occurred. People pass stool in the fields and most probably some carrier has passed the organism in the field and rain water contaminated the source of drinking water¹³.

In year 2004 (June-November), eight hundred twenty three cases of diarrheal disease and 12 deaths (1.45%) were reported in different districts of Balochistan province from June to November 2004¹³.

The most serious outbreak was in village of Badree in Thesil Wadh, district Khuzdar. where in three days 148 cases of severe gastroenteritis and 4 deaths (2.7%) occurred. These four patients were two females one aged 60years and one female child of 20months one male age 45years and one male child age 18months. All cases were from same village using a common source of water.

It is the first outbreak which occurred due to serotype Inaba which spread quickly but the mortality rate was low (0.4%) as compared to *Vibrio cholera* 0139 (3.6 %) and *Vibrio cholerae* Ogawa (2.7%) Probably people living in this area developed some immunity against "Ogawa" strains but the Inaba sero-type was new one against which people had no immunity. There for it spread very quickly

CONCLUSION

Vibrio cholerae O1, Inaba El Tor, strain was isolated as etiological agent for severe diarrheal disease outbreak, which was spread through drinking water. The mortality rate in this outbreak was 0.4%. Most probably the source

of drinking water was contaminated due to rain in this area. The isolated strain is new pathogen in this area for severe diarrheal disease outbreaks. The strategy applied for prevention of disease was successful and outbreak was controlled effectively and no further cases were reported. After the isolation of serotype "Inaba" it can be said that all three serotypes namely Ogawa, Inaba and 0139 are prevalent in Balochistan.

RECOMMENDATIONS

It is recommended that in case of severe diarrheal outbreaks *Vibrio cholerae* must be considered as most common etiological agent.

The drinking water source must be protected by stool contamination, especially in case of spring water, the catchments area to be prohibited for human stool contamination.

Ampicillin, Ciprofloxacin, Tetracycline, or Doxycycline can be used affectively in such outbreaks.

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PREVIOUS RELATED STUDIES



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