

'GASTRO' OF MIRPUR KHAS (SINDH) IS RESOLVED

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Background: Diarrhoeal disease is a common cause of major public health concern in many parts of the world including Pakistan. **Methods:** Eighteen stool samples were received from Civil Hospital Mirpur Khas (Sindh). All the specimens were processed for culture and antimicrobial susceptibility according to Clinical Laboratory Standards Institute (CLSI) guidelines. **Results:** Eight out of eighteen (44.4%) samples were positive for *Vibrio cholerae*. The isolates were gram negative rods, showed darting motility and were Oxidase positive. Contact with distilled water immobilized all these strains (Mdw). API 20NE was used for the biochemical identification and serological confirmation was done with 'difco' antisera. Kirby-Bauer disc diffusion method was performed for their respective susceptibility to various antibiotics. All these isolates were confirmed to be *Vibrio cholerae* O1 Biotype El Tor Serotype Ogawa. The isolates were generally sensitive to majority of the antibiotics but resistant to nalidixic acid except one strain. Six out of eight isolates were resistant to co-trimoxazole. **Conclusion:** Cholera must be suspected in outbreaks of such kind of 'gastro'.

Key words, Diarrhoea, Gastroenteritis, Mirpur Khas, Cholera, Mdw (motility in distilled water)

INTRODUCTION

'Gastro' is a common English language prefix derived from the ancient Greek 'gastros' which refers to stomach. The term 'Gastro' has probably been coined for a condition encompassing vomiting, diarrhoea and abdominal pain etc. Ambiguous as it seems, 'gastro' neither implies a specific disease nor does it identify any definite etiological agent(s). The term has been heard in the media in Pakistan for the last couple of years referring to hundreds of cases with abdominal pain, vomiting and diarrhoea admitted in various hospitals.

Cholera has been responsible for seven pandemics worldwide.¹ The first started in 1817 in India and spread to Europe. The current 7th pandemic began in Indonesia in 1961 and spread through out the world.²

The first six pandemics of cholera which occurred in the Indian subcontinent and subsequently in other areas of the world between 1817 and 1923 are said to be caused by the classical biotype.³ *V. cholerae* O1 biotype El Tor was first reported in 1905.⁴ However, *V. cholerae* O1 biotype El Tor displaced *V. cholerae* O1 classical biotype in the seventh pandemic of cholera.¹ Biotyping and serological testing of the positive isolates in our study confirmed involvement of *Vibrio cholerae* O1 El Tor serotype Ogawa. This strain is widely recognized as being responsible for outbreaks of cholera.⁵ Regular isolation of *Vibrio cholerae* in our country suggests that *Vibrio cholerae* El Tor Ogawa is endemic in our country.^{6,7} One such episode occurred in Mirpur Khas (Sindh) around 10th of May, 2008. This attracted attention of University of Health Sciences, Lahore to take the initiative and investigate as to what could be

the possible etiological agent of this so called 'gastro'.

MATERIALS AND METHODS

This study was prompted by news items appearing in the electronic and print media of Pakistan. The national press had reported occurrence of 'Gastro', in interior Sindh affecting hundreds of people. The Department of Microbiology, University of Health Sciences, Lahore took an initiative to identify the cause of this 'gastro' in Mirpur Khas. The study design was descriptive-case series.

Eighteen stool samples from cases of 'gastro' admitted in Civil Hospital Mirpur Khas, (Sindh) were received in buffered glycerol saline. Stool samples from patients ranging from eleven months to thirty years of age, suffering from vomiting, diarrhoea with moderate to severe dehydration were studied.

The stool samples were initially enriched in tetrathionate broth and alkaline peptone water (pH 8.6). Each sample after 12–18 hours of enrichment in tetrathionate broth and 6–8 hours in alkaline peptone water was inoculated on blood agar, MacConkey agar, DCA (Deoxycholate citrate agar) and TCBS (Thiosulphate citrate bile salt sucrose agar). The plates were incubated at 35 °C for 18–24 hours. After incubation the plates were examined for the presence of lactose fermenters (*Escherichia coli*) and non-lactose fermenters (*Salmonella*, *Shigella* and *Vibrio*).

Yellow sucrose fermenting colonies from TCBS, β-hemolytic from blood agar and pale colonies from MacConkey agar were gram stained and their motility seen by hanging drop preparation.

The suspected isolates were purified on blood agar and subjected to oxidase and catalase. API 20NE galleries (Biomeriux UK) were used for the

biochemical identification. Distilled water immobilization was also performed. Final confirmation was carried out by using polyvalent and monovalent antisera (BD difco).

String test, Beta-haemolysis on sheep blood agar, CAMP test, sensitivity to polymyxin B (300 IU), agglutination of chicken red cells and Voges-Proskauer test were performed to determine the biotype.

Antimicrobial susceptibility testing was performed by Kirby-Bauer Disc diffusion method using antibiotic discs (Oxoid UK) as per the CLSI guidelines.

RESULTS

Eight out of eighteen (44.4%) specimens were positive for *Vibrio cholerae*. Results of biochemical profile, biotyping of the isolates are given in Tables 1 and 2.

All these eight isolates reacted with polyvalent and monovalent Ogawa antisera and did not react with Inaba. All the eight isolates were *Vibrio cholerae* Serotype O1 Ogawa biotype El Tor.

Antibiotic sensitivity results for these eight strains are given in Table-3. All isolates were generally sensitive to most of the antibiotics and

resistant to nalidixic acid except strain #17. Six out of eight strains were sensitive to co-trimoxazole.

Table-1: Biochemical profile of the 8 isolates

S #	Gram stain	Motility	Mdw	Ox	Growth on TCBS
05	Gram -ve rods	Darting	Imb	+	Y
06	Gram -ve rods	Darting	Imb	+	Y
09	Gram -ve rods	Darting	Imb	+	Y
10	Gram -ve rods	Darting	Imb	+	Y
12	Gram -ve rods	Darting	Imb	+	Y
14	Gram -ve rods	Darting	Imb	+	Y
15	Gram -ve rods	Darting	Imb	+	Y
17	Gram -ve rods	Darting	Imb	+	Y

Mdw: Motility in distilled water, Imb: Immobilized, Ox: Oxidase
Y: yellow colonies

Table-2: Biotyping of the 8 isolates

S#	ST	β-H	CAMP test	PB sens. (300 IU)	Chicken RBCs
05	+	+	+	R	+
06	+	+	+	R	+
09	+	+	+	R	+
10	+	+	+	R	+
12	+	+	+	R	+
14	+	+	+	R	+
15	+	+	+	R	+
17	+	+	+	R	+

ST: String test, β-H: beta-haemolysis on sheep blood agar,
PB sens: sensitivity to 300 IU polymyxin B, Chicken RBCs agglutination

Table-3: Antimicrobial Susceptibility Pattern

S#	AK	AMP	ATM	CAZ	CRO	CXM	C	SXT	IPM	LEV	MXF	NA	OFX	TET
05	S	S	S	S	S	S	S	R	S	S	S	R	S	S
06	S	S	S	S	S	S	S	R	S	S	S	R	S	S
09	S	S	S	S	S	S	S	R	S	S	S	R	S	S
10	S	S	S	S	S	S	S	R	S	S	S	R	S	S
12	S	S	S	S	S	S	S	S	S	S	S	R	S	S
14	S	S	S	S	S	S	S	R	S	S	S	R	S	S
15	S	S	S	S	S	S	S	R	S	S	S	R	S	S
17	S	S	S	S	S	S	S	S	S	S	S	S	S	S

S: sensitive, R: resistant, AK: Amikacin, AMP: Ampicillin, ATM: Aztreonam CAZ: Ceftazidime, CRO: Ceftriaxone, CXM: Cefuroxime, C: Chloramphenicol, SXT: Cotrimoxazole, IPM: Imipenem, LEV: Levofloxacin, MXF: Moxifloxacin, NA: Nalidixic acid, OFX: Ofloxacin, TET: Tetracycline

DISCUSSION

The term 'Gastro' is a misnomer and has been used by the national press to imply gastroenteritis. However, it is important to realize that infectious gastroenteritis can be caused by a number of bacterial, viral or parasitic agents. In order to provide a comprehensive management of infections, proper identification of the causative agent is absolutely critical. This is particularly crucial when a major outbreak of gastroenteritis occurs.

Despite the reported outbreak of gastro in Mirpur Khas, involving paediatric and adult cases alike, no comprehensive management strategy was probably devised. At the time of this study, it was gathered that 138 cases were admitted in the Civil Hospital, Mirpur Khas and were being empirically treated with Gentamicin and Ampicillin. Similar

outbreaks had been reported on a number of occasions by the national press in the recent past. Probably no concrete efforts had been made to identify the causative agent.

Vibrio cholerae was detected in 8 out of 18 stool samples in one such outbreak in Mirpur Khas. Out of the 18 cases investigated, majority were of paediatric age. It is worth noting that these were the only 8 samples in which the transport medium remained unaltered. Rest had turned acidic, the reaction which is inhibitory to the viability of the *Vibrio*. A rapid transport of the specimens could have resulted isolation of *Vibrio* in the remaining samples also. The reported clinical presentation of patients was also consistent with cholera infection as per the telephonic information rendered.

Regular isolation of *Vibrio cholerae* in our country suggests that *Vibrio cholerae* El Tor Ogawa

is endemic in our country.^{6,7} The present study provides similar findings. *Vibrio cholerae* O1 El Tor has also been implicated in outbreaks of cholera in the neighbouring countries.⁸ Moreover, the positive cultures in our study were uniformly resistant to Nalidixic acid, a finding which has also been reported by others also.⁹

Improvements in water supply, sanitation, food safety and community awareness of preventive measures are the best means of preventing cholera, as well as other diarrhoeal diseases.

CONCLUSION

Cholera is endemic in many regions of the world and must be strongly suspected when episodes of this type occurs in our settings. Laboratory facilities for identification of *Vibrio cholerae* must be established in those areas where infections show more endemicity.

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