Original Article

CHANGING EPIDEMIOLOGY AND SENSITIVITY PATTERN OF *VIBRIO CHOLERAE* AT RAWALPINDI

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ABSTRACT

Objective: To establish the serotypes of *Vibrio cholerae* (VC*)* endemic in our set up and to determine the sensitivity pattern of the organism.

***Methods*:** The study was carried out on 123 isolates of *Vibrio cholerae* isolated from stool samples of patients of diarrhea. Samples were collected in plain containers/alkaline peptone water. Inoculation was done on TCBS agar, McConkey's agar and Salmonella Shigella agar. Identification of the organisms was based on, sucrose fermentation on TCBS agar a positive oxidase test and biochemical profile by API 20 E galleries. Serological confirmation was done by standard antisera. Biotypes were confirmed by Voges-Proskauer (vp) reaction, Polymixin B (50 i.u) sensitivity and sheep red cell haemolysis. Antibiotic sensitivity was carried out against Ampicillin,Tetracycline, Trimethoprim/Sulphamethoxazole, Erythromicin, Nalidixic acid, Ofloxacin and Ciprofloxacin by Kirby Bauer disc diffusion technique.

***Results*:** A total of 123 isolates recovered during the period 1997-2002 were studied. All strains belonged to sero type O1. Till 1998 all strains were of biotype EL Tor. Classical biotype appeared in 1999 and remained the dominant variety during 2000-2001. Year wise comparison of the antimicrobial sensitivity pattern shows that resistance to Nalidixic acid emerged in 1999 and is uniformly continuing whereas resistance to Ampicillin and Tetracycline has fallen to very low levels. No isolate was resistant to the fluoroquinolones.

***Conclusion:* 1**. *Vibrio cholerae* O1 is endemic in Rawalpindi.

2. EL Tor was the biotype causing epidemics predominantly in our set up till 1998. Now the Classical variety has emerged and both types are probably co-existing.

3. All the isolates in our study were completely resistant to Nalidixic acid but sensitive to the fluoroquinolones and Erythromycin. Ampicillin and Tetracycline which were earlier resistant have reverted to sensitive.

KEY WORDS: *Vibrio cholerae*, biotype, serotype, antibiotic sensitivity.

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INTRODUCTION

Cholera and resembling diseases have existed in Europe and Asia since ancient times. The disease which we now recognize as cholera caused by *vibrio cholerae* O1 can be traced back to the early 19th century, affecting a large population of the world through seven pandemics. The first started in 1817 in India and spread to Europe. The seventh began in Indonesia in 1961 and passed through Bangladesh and India in 1963 & 1964. By 1970, it reached Africa and Europe. It spread across the Atlantic to the South and Central America by 1992. This is considered to be the most widespread and the longest in duration so far. This pandemic in contrast to the others was caused by the EL Tor biotype.[1] Superimposed on this pandemic, an epidemic started due to a differ-

ent strain serologically classified as *Vibrio cholerae* O 139. It started from Madras India in 1992[2] and through Calcutta reached Dacca, Bangladesh by 1993[3] spreading out to Thailand and Karachi, Pakistan the same year.[4] It also involved China, Nepal and Malaysia and a few imported cases were also reported in Germany, UK and USA.[1]

Pakistan after the separation of Bangladesh was not considered an endemic area of Cholera. It remained free from this disease till 1976. From this year onwards *Vibrio cholerae* has been isolated in our laboratories quite frequently so it can be assumed that it has been introduced in the environment by the influx of refugees in 1976/77.[5]

Antibiotic resistance was rare among *Vibrio cholerae* isolates before 1976; they were sensitive to Tetracycline (tet), Ampicillin (amp), Cholramphenicol (cap), and Nalidixic acid (nal). Subsequently varying levels of resistance against these antibiotics have been reported.[5,6]

We present a study based on data from 1997 to 2002 collected at the Department of Microbiology, Army Medical College, Rawalpindi to review the existing situation with regards to the epidemiology and the antimicrobial sensitivity of the organism.

MATERIALS AND METHODS

A total of 123 isolates of *Vibrio cholerae* were studied. They were isolated from stool samples of patients suffering from diarrhoea or suspected of cholera received routinely in the Microbiology Department of Army Medical College, Rawalpindi. The stool samples were collected in plain plastic containers/alkaline peptone water. Cultures were set up on Thiosulphate Citrate Bile salt Sucrose (TCBS) agar, McConkey's agar and Salmonella Shigella agar. Sucrose fermenting colonies on TCBS agar and non lactose fermenting colonies on the other two media were further identified by a positive oxidase test and confirmed biochemically using API 20-E galleries. Serological confirmation was done by antisera from Murex Biotech (Dartford, England). Biotype was determined by Voges-Proskauer reaction, Polymixin B (50 i.u) sensitivity and sheep red cell haemolysis.

Antibiotic sensitivity testing was carried out by Kirby Bauer disc diffusion technique. The antibiotics tested were Ampicillin (amp) Tetracycline (tet), Trimethoprim/Sulphamethoxazole (tmp/sxt), Erythromycin (ery), Nalidixic acid (nal), Ofloxacin (ofl) and Ciprofloxacin (cip).

RESULTS

A total of 123 isolates recovered during the period 1997-2002 were studied. All belonged to sero type O1; no sero type O139 was isolated. Till 1998 all strains were of biotype 'EL Tor'. 'Classical' biotype appeared in 1999 and remained the dominant variety during 2000-2001 (table-I). Year wise comparison of the antimicrobial sensitivity pattern is presented (table-II). Resistance to nal emerged in 1999 and is uniformly continuing whereas resistance to amp and tet has fallen to very low levels. No isolate was resistant to fluoroquinolones.

Table-I: Year wise distribution of Serotypes and biotypes of *Vibrio cholerae* (n=123)

Year	*Serotype*			*Biotype*	
	O1	*O139*	*Non O1*	*ELTor*	*Classical*
1997	24	_	_	24	
1998	16	_	_	16	
1999	31	_	_	20	11
2000	23	_	_	01	22
2001	24	_	_	_	24
2002	05	_	_	05	

Table-II : Year wise comparison of antimicrobial sensitivity of *Vibrio cholerae* (n=123)

Drug	*1997*		*1998*		*1999*		*2000*		*2001-2002*	
	S	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*
AMP	2(8.34%)	22(91.66%)	13(81.25%)	3(18.75%)	31(100%)	-	23(100%)	-	23(95.83%)	1(4.17%)
SXT		24(100%)	_	16(100%)	_	31(100%)	_	23(100%)	1(4.17%)	23(95.8%)
ERY	24(100%)	_	15(93.75%)	1(6.25%)	31(100%)	_	23(100%)	_	24(100%)	_
TET	2(8.70%)	21(91.30%)	4(25%)	12(75%)	31(100%)	_	19(82.60%)	4(17.40%)	24(100%)	_
NAL	24(100%)	_	16(100%)		_	31(100%)	_	23(100%)	_	24(100%)
OFL	24(100%)	_	16(100%)		31(100%)		23(100%)		24(100%)	
CIP	24(100%)	_	16(100%)		31(100%)		23(100%)		24(100%)	

DISCUSSION

Regular isolation of *Vibrio cholerae* in our laboratory and other laboratories of Pakistan suggests that *Vibrio cholerae* EL Tor Ogawa is endemic in our country.[4,5,6] Poor sanitary conditions, overcrowding , with a majority of the people living below the poverty line, lack of proper sewage disposal system and improper water supply, all provide happy hunting ground for this organism once it is introduced in a community. Thus small epidemics can be expected.

Vibrio cholerae O1 is the only serotype isolated from Rawalpindi. V*ibrio .cholerae* O139 has not been isolated so far.[5,6] In our study too, no O139 strain was isolated. Outbreaks due to *Vibrio Cholerae* O1 and O139 have been reported from Karachi in 1993-94 but O139 strain disappeared by 1996.[4] *Vibrio Cholerae* O139 was also reported by Pirkani et al from Balochistan as the causative organism of a diarrhoeal epidemic affecting the population of Sibi and surrounding area in September 1994. There were 110 reported cases including 4 deaths. The organism was isolated from the stools of patients as well as from the sources of drinking water[7].

ElTor was the prominent biotype reported from Pakistan by Karamat et al in 1988, 1990, 1994and Mubasher et al in 1998[5,6,8,9]. Findings of our study reflect that the same was true till 1998. In 1999 35.48% of isolates belonged to the 'Classical' biotype. In the years 2000 and 2001 all isolates were of 'Classical' variety. In the year 2002 five V*ibrio cholerae* were isolated and all of them were of ELTor biotype.

No significant antimicrobial resistance was reported from Rawalpindi- Islamabad till 1990 when Karamat et al reported 35% resistance against tmp/sxt 21% to Ampicillin and 10% to Nalidixic acid[5]. The same workers reported 6% resistance to Ampicillin, 91% to tmp/sxt, 80% to Chloramphenicol and 91% to Tetracycline in 1993. No resistance to quinolones was reported[6]. In 1998 at Karachi, Nizami et al reported high degree of resistance to Tetracycline, Ampicillin, and Erythromycin but complete sensitivity to quinolones including Nalidixic acid and cephalosporins[10]. Another study from Dow Medical College Karachi reflected 98% sensitivity to Nalidixic acid, 100% to fluoroquinolones, 86% to Doxycycline and complete resistance to tmp/sxt[11]. Our study reflects high degree of resistance to Ampicillin in 1997 (91.96%) then falling in the subsequent years to 4.17% in 2001. Resistance to tmp/sxt has varied between 100% to 95.8%. An alarming change was seen with Nalidixic acid. All isolates of *Vibro Cholerae* were fully sensitive to this drug till 1998. From 1999 onwards till 2002 not a single isolate has been found sensitive to the drug. All isolates were however sen-

sitive to Erythromycin and to both the fluoroquinolones tested.

Antimicrobial resistance in *Vibrio cholerae* has been reported earlier from other countries like Tanzania in 1979 and Somalia in 1983.[12,13] Resistance to Furazolidine and tmp/sxt has been reported in Calcutta, India by Sengupta et al. in the year 2000.[14] Nalidixic acid resistance was reported from Calcutta in the year 2001. This study has shown uniform sensitivity to Tetracycline, Chloramphenicol, Ofloxacin and Ciprofloxacin but resistance to Ampicillin, Furazolidine and tmp/sxt[15]. Nalidixic acid resistance has also been reported from Kazan city Russia in 2002[16].

CONCLUSION

1. Cholera is endemic in Pakistan. *Vibrio cholerae* O1 "ELTor" is the predominant type. Classical variety surfaced in 1999 and by 2001 had replaced "ELTor" but the later serotype has re-emerged. Studies in the coming years will tell whether they co-exist or one replaces the other.
2. *Vibrio cholerae* O139 has not so far been introduced in the environment at Rawalpindi.
3. Antimicrobial sensitivity varies from region to region and also changes with time within the same region. Presently at Rawalpindi the existing strains have reverted sensitivity to Ampicillin and Tetracycline, but have become completely resistant to Nalidixic acid. Erythromycin, Ofloxacin and Ciprofloxacin however, are still fully effective.

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