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Spectrum of multiple antibiotic resistance among clinical strains of *Vibrio cholerae* O1 and O139 isolated during 1999-2003 in Orissa, India

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ABSTRACT

Antimicrobial susceptibility pattern of 472 *Vibrio cholerae* O1 and 156 O139 strains isolated from diarrhoea patients from 1999-2003 in Orissa, India were analyzed to determine the changing trends. Ampicillin and neomycin resistance decreased from 1999 (96.2 and 88.8% respectively) to 2003 (88.2 and 49.7% respectively) among O1 serogroup. Resistance to cotrimoxazole, furazolidone, nalidixic acid and streptomycin was observed to be almost constantly high among O1. Both the serogroups showed development of resistance to ciprofloxacin, norfloxacin and tetracycline; remained largely susceptible to gentamicin and tetracycline in each year. *V. cholerae* O139 strains exhibited fluctuating trends of susceptibility to ampicillin, chloramphenicol and cotrimoxazole. Nalidixic acid resistant O139 was emerged in 1999 and all became resistant from 2000 onwards. Pearson's Chi-square analysis revealed that the serogroup of O1 and O139 exhibited increase and decrease of resistance to almost all the antibiotics within one or two years of interval. Association of multiple drug resistance even up to 9 drugs including fluoroquinolone was observed with constant change in drug resistance profile. To our belief it is the first report to be observed from the analysis that *V. cholerae* O1 can carry the multi drug resistance longer than O139 strains. It is suggested to identify the susceptibility pattern of *V. cholerae* to determine the effective drug for the treatment of cholera.

Key Words: Antibiotics, Cholera, Diarrhoea, Multiple drug resistance, Susceptible, *Vibrio cholerae* O1 and O139.

INTRODUCTION

Cholera is caused by strains of *Vibrio cholerae* belonging to serogroup O1 and O139 associated with toxin mediated massive outpouring of electrolytes-rich isotonic fluids pass into bowels leading to volume depletion and shock. Rehydration therapy through intravenous or oral routes markedly decreases case fatality rates[1]. Although antimicrobial therapy has not proven useful in prevention[2], it can reduce the total volume of stool passed and shorten both the duration of diarrhoea and the period of faecal excretion of *V. cholerae*[3]. Antibiotics are therefore recommended for all cases of suspected cholera with severe dehydration as a useful adjunct to fluid and electrolyte replacement, which remains the principal focus for treatment[4]. However the emergence of drug resistance during the past several years have become a major concern in the therapeutic field; whatever the cause may be. In recent years several multi-drug resistances (MDR)s have been characterized in

both gram negative and gram positive bacteria including *E. coli*[5], *Staphylococcus aureus*[6], *Shigella dysenteriae* Type-1 infection in Africa[7], *Salmonella typhi* in India[8] and *V. cholerae* in Ecuador[9]**.** The resistance pattern of clinical strains of *V. cholerae* O1 and O139 to commonly prescribed antibiotics is increasing both in developing as well as in developed countries. Resistance has emerged even to newer more potent antimicrobial agents. It is essential to observe the variations in resistance and to relate these variations to mechanism of resistance. We have been monitoring the antibiogram pattern of *V. cholerae* among cholera patients for the last one decade in Orissa, India[10-11]. The major objective of this study was to analyze the resistance pattern of antibiotics and their shifting trends among clinical strains of *V. cholerae* isolated between 1999-2003 in Orissa, an eastern state of Indian subcontinent.

MATERIAL AND METHODS

Bacteriology and serogrouping

Thiosulfate-citrate-bile salt sucrose agar (TCBS, Eiken, Tokyo, Japan) was used as the selective medium for the isolation of *V. cholerae*. Individual rectal swabs collected from diarrhoea patients were inoculated on TCBS plate and then streaked for colony isolation. The inoculated plates were incubated at 37°C for 18-24 h and subsequently examined for the growth of *V. cholerae.* A multitest medium was used for presumptive identification of *V. cholerae*[12]. Serogrouping of *V. cholerae* was done using growth from the multi-test medium with polyvalent O1 and mono-specific Inaba and Ogawa antisera (Difco, USA). *V. cholerae* strains which did not agglutinate with the O1 antiserum were checked with monoclonal O139 antiserum developed at NICED, Kolkata[13]. Original stock cultures of isolates were kept in 20% glycerol Luria-Bertani broth at -70°C.

V. cholerae *strains*

All the *V. cholerae* strains used in this study were of clinical origin. A total of 628 strains of *V. cholerae* examined were isolated during the period from 1999 to 2003 from hospitalized diarrhoea patients. Of the two serogroups, *V. cholerae* O1 was the dominant one causing cholera outbreaks. The incidence of O139 on the other hand became less than O1 and its isolation rate gradually decreased from 2001 and became zero during 2003. Of the total, 472 and 156 were *V. cholerae* O1 and O139 respectively and all the strains were included in this study for the analysis of drug resistance pattern.

Antimicrobial susceptibility

Antimicrobial susceptibility test of all the strains of *V. cholerae* O1 and O139 from each year of the study was done by disc-diffusion method[14] with commercial discs (Hi Media, Bombay, India). The antibiotics used were ampicllin (A, 10mcg), chloramphenicol (C, 30mcg), co-trimoxazole (Co, 25mcg), ciprofloxacin (Cf, 5mcg), furazolidone (Fz, 100mcg), gentamicin (G, 10mcg), neomycin (N, 30mcg), nalidixic acid (Na, 30mcg), norfloxacin (Nx, 10mcg), streptomycin (S, 10mcg) and tetracycline (T, 30mcg). Characterization of strains as susceptible or resistant was based on size of the inhibition zone around each disc according to the manufacturer's instruction, which matched interpretive criteria recommended by WHO[4]. Strains showing an intermediate zone of inhibition were interpreted as resistant to that drug on the basis of previous MIC studies conducted with *V. cholerae*[15].

Statistical analysis

For comparing the differences in resistance of each drug between two successive years, the duration of 4 years from 1999 to 2003 were divided into seven groups for O1 and 5 groups for O139 (No *V. cholerae* O139 was isolated in 2003). Significant differences in increase or decrease of resistance of each drug between two consecutive years were calculated based on Pearson's Chi-square values. A *'P'* value of < 0.05 was considered as statistically significant.

RESULTS

The results on drug resistance pattern of *V. cholerae* O1 and O139 isolated during the study period are exhibited in Table-1. Interesting, fluctuations in drug resistance pattern between the strains of *V. cholerae* O1 and O139 serogroups and among the strains of each serogroup were observed. Most importantly development of resistance to norfloxacin and ciprofloxacin was observed by both the serogroups.

Table 1: Resistance to different antibiotics and statistical analysis of significant changes in drug resistance (either increase or decrease) between years among V. cholerae O1 and O139

Drugs	Resistance of V. cholerae							P-value					
	V. cholerae	1999 % n=54 n=29	2000 % n=78 n=72	2001 % n=56 n=32	2002 % n=71 n=23	2003 % n=213 n=00	1999 vs 2000	1999 vs 2001	2000 vs 2001	2000 vs 2002	2001 vs 2002	2001 vs 2003	2002 vs 2003
Ampicillin	O1	52(96.2)	29(37.1)	35(62.5)	45(63.3)	188(88.2)	<0.0001**	<0.0001**	0.0007*	0.0004*	1. 00	<0.0001*	<0.0001*
	O139	29(100)	19(26.3)	32(100)	15(65.2)		<0.0001**	NC	<0.0001*	<0.0001*	<0.0001**		
Tetracycline	O1	3(5.5)	23(29.4)	9(16.1)	3(4.2)	8(3.7)	<0.0001*	0.0192 *	0.0414**	<0.0001**	0.0081**		
	O139	5(17.2)	3(4.1)	14(43.7)			0.0046**	<0.0001*	<0.0001*	0.1212	<0.0001**	0.0081**	1.00
Chloramphenicol	O1	6(11.1)	29(37.1)	20(35.7)	13(18.3)	117(54.9)	<0.0001*	<0.0001*	1.00	0.0041**	0.0065**		
	O139	14(48.2)	22(30.5)	6(18.7)			0.0135**	<0.0001**	0.0718	<0.0001**	<0.0001**	0.01040*	<0.0001*
Gentamicin	O1	2(3.7)	15(19.2)	7(12.5)	3(4.2)	25(11.7)	<0.0014*	0.0398*	0.2408	0.0014**	0.0398**		
	O139		9(12.5)	6(18.7)			0.0002*	<0.0001*	0.335	0.003**	<0.0001*	1.00	0.0652
Co-trimoxazole	O1	54(100)	75(96.1)	56(100)	64(90.1)	213(100)	0.1212	NC	0.1212	<0.0001**	<0.0001**		
	O139	9(31)	22(30.5)	30(93.7)	15((65.2)		1.00	<0.0001*	<0.0001*	<0.0001*	<0.0001*	NC	<0.0001*
Furazolidone	O1	54(100)	76(97.4)	56(100)	71(100)	213(100)	0.2462	NC	0.2462	0.2462	NC		
	O139	29(100)	54(75)	30(90.7)	15(65.2)		<0.0001**	0.0015**	0.0085*	0.1646	<0.0001**	NC	NC
Nalidixic acid	O1	53(98.1)	73(93.5)	56(100)	67(94.3)	213(100)	0.3311	0.1212	0.0140*	1. 00	0.0289**		
	O139	5(17.2)	72(100)	32(100)	23(100)		<0.0001*	0.0001*	NC	NC	NC	NC	0.0289*
Neomycin	O1	48(88.8)	59(75.6)	30(53.5)	51(71.8)	106(49.7)	0.0246	<0.0001**	0.0018**	0.6289	0.0125*		
	O139	24(82.7)	36(50)	30(93.7)	7(30.4)		<0.0001**	0.0249**	<0.0001*	0.0059**	<0.0001**	0.6712	0.0022**
Streptomycin	O1	54(100)	50(64.1)	47(83.9)	67(94.3)	213(100)	<0.0001**	0.0001**	0.0020*	<0.0001*	0.040*		
	O139	26(89.6)	46(63.8)	26(81.2)	7(30.4)		<0.0001*	0.107	0.0016*	<0.0001**	<0.0001**	<0.0001*	0.0089*
Ciprofloxacin	O1	34(62.9)	34(43.5)	23(41)	6(8.4)	47(22)	0.007**	0.0029**	0.7749	<0.0001**	<0.0059**		
	O139	4(13.7)	28(38.8)	20(62.5)			0.0009*	<0.0001*	0.0018*	<0.0001**	<0.0001**	0.0059**	0.0092*
Norfloxacin	O1	9(16.6)	12(15.3)	7(12.5)	3(4.2)	47(22)	0.8473	0.4222	0.6796	<0.0140**	0.0892		
	O139	3(10.3)	12(16.6)	20(62.5)			1.00	<0.0001*	<0.0001*	<0.0001**	<0.0001**	0.0892	0.0002*

*: Statistically significant increase in resistance to the antibiotic in question between the years of comparison, **: statically significant decrease in resistance to the antibiotic in question between years of comparison and NC: not comparable data.

The incidence of ampicillin resistant *V. cholerae* O1 was decreased during 2000-2002 in comparison to 1999 and again increased in 2003. Almost all the strains of *V. cholerae* were highly resistant to streptomycin, furazolidone and nalidixic acid throughout the study period except *V. cholerae* O139 with low resistance to streptomycin (30.4%) in 2002 and nalidixic acid (17.2%) in 1999. *V. cholerae* O1 revealed hike in resistance to chloramphenicol in 2000 compared to the level in 1999 and attained its peak in 2003 through fluctuating trends. Maximum (88.8%) neomycin resistant *V. cholerae* O1 was observed in 1999, which decreased in subsequent period through wavering trend. Highest, 29.4% tetracycline resistant *V. cholerae* O1 was detected in 2000 in comparison to other years. Ciprofloxacin and norfloxacin resistant *V. cholerae* O1 were encountered in each year in varying proportion. Highest 62.9% ciprofloxacin and 22% norfloxacin resistant

Table 2: Different antibiotics resistance profile among *V. cholerae* O1 & O139 strains in Orissa.

	Year/ serogroup									
	1999		2000		2001		2002		2003	
Antibiotic Profile	**O1**	**O139**	**O1**	**O139**	**O1**	**O139**	**O1**	**O139**	**O1**	**O139**
ACoFzNNaSCf	21								14	
ACoFzNNaS	10						29		14	
ACoFzNaS	2				17				33	
ACoFzNNaSNxCf	5		3							
CoFzNaS	1		2	2	1		9		11	
ACoFzNaSC					5		6		2	
ACoFzNaSCNx									8	
ACoFzNaNSC	5		6						47	
CoFzNaNS			6				9			
CoFzNaN			6							
AFzNaNSNx				5						
SNa				8						
AFzNaNSCf				8						
ACoFzNaNSCNxCf	1	2							3	
CoFzNaNSCf			6		1					
ACoFzNaNSTCf	2				4					

V. cholerae O1 were encountered in 1999 and 2003 respectively, which appeared in varying proportion in other year.

Variable trends of drug resistance pattern were exhibited by the strains of *V. cholerae* O139 during the years of study period. Yearly increase and decrease of resistance to ampicillin was observed by O139 strains. The strains of O139 were found mostly resistant to nalidixic acid and furazolidone and susceptible to tetracycline, chloramphenicol, norfloxacin, ciprofloxacin and gentamicin. The incidence of co-trimoxazole resistant strains of O139 was found highest in 2001 in comparison to other years. The frequency of ciprofloxacin and norfloxacin resistant strains of O139 was observed highest during 2001; where as a contrast scenario was observed in 2002, being all O139 strains exhibiting susceptibility. Nalidixic acid resistant strains of O139 were found 17.2% in 1999 and 100% in 2000, 2001 and 2002.

The statistical analysis revealed yearly increase or decrease of resistance to tested drugs by *V. cholerae* strains is shown in Table-1. The comparison of each drug in a year with two

successive years mostly exhibited significantly increasing or decreasing resistance pattern by both the strains of O1 and O139.

Antimicrobial resistance pattern of O1 and O139 strains revealed that most of the strains were multidrug resistant and pass through various shifting. More number of *V. cholerae* strains isolated had high rates of resistance to various antibiotics from 4 up to 9 antibiotics tested with different combination in each year (Table-2). The retention of drug resistance varies yearly exhibiting an unstable phenotypic character. Instead of a particular type, different combination of drug resistance was marked. In our study some strains of *V. cholerae* were observed to be resistant to more than two antibiotics with different combination in each year. It is interesting that some strains of O1 were resistant to highest number of antimicrobial resistance profile like ACoFzNaNSCNxCf. Again some group of MDR strains disappeared for consecutive one or two year and again appeared with the same combination of drug resistance profile. It was observed that antibiotic resistance character carried by the strains of O1, continued to exist for long period although disappear for a particular period of time. As shown in Table-2, some of the major drug resistance profiles which are recorded among *V. cholerae* O1, disappeared for one or two consecutive years and again appeared. The dominant profiles like ACoFzNNaSCf followed by ACoFzNNaS are recorded among *V. cholerae* O1 strains in 1999 and disappeared for three and two years respectively. Similarly the profile ACoFzNaS reported among *V. cholerae* O1 in 1999 became the dominant drug resistant profile in 2001 and 2003 after being disappeared during 2000 and 2002 respectively. The drug resistance pattern AFzNaNSNx, SNa and AFzNaNSCf among *V. cholerae* O139 are recorded once in 2000 and remained quiescence for the previous and next successive years. The drug resistance profile ACoFzNaNSCNxCf and CoFzNaS were encountered among both the serogroups in 1999 and 2000 respectively and disappeared from among O139 strains during subsequent period. But CoFzNaS resistance was observed every year after 1999 and again reappeared in 2003 after a quiescence period of three years.

DISCUSSION

Past few years have encountered various shifting trends in antibiotic resistance profile among O1 strains with an increasing order and ACoFzNaNSCNxCf resistant O1 disappeared due to enhanced mobility of the genetic element associated with drug resistance plasmids of *V. cholerae* O1[16] and O139; which have led to a major concern in developing countries where the incidence of cholera is high. In the present 4 years study, the antibiotic pattern revealed continuous fluctuations within the same serogroup in different time period. The tale of nalidixic acid resistant O139 was similar to the counterpart *V.cholerae* O1 strains. Since 1992, the susceptibility of nalidixic acid by O139 was presumed to be as marker to distinguish between O1 and O139; while O1 was completely resistant to nalidixic acid. During 1999, O139 started its resistance to nalidixic acid in Kolkata and progressively increased in other part of India. In our study all the strains of O139 were found to be resistant to nalidixic acid in 2000. Ciprofloxacin has been regarded as a first line drug of choice and effective alternative to combat the multidrug resistant enteric and other pathogens. The emergence of ciprofloxacin resistant *V. cholerae* in India and in other part of the world has been extensively reviewed[17-18]. In our study incidence of ciprofloxacin resistant *V. cholerae* O1 and O139 were reported in the year 1999 onwards in variable proportion**.** Norfloxacin on the other hand is widely prescribed to chase the tetracycline resistant *V. cholerae* causing cholera outbreaks. Incidence of norfloxacin resistant *V. cholerae* was encountered in various parts of the world including India[17, 19]. We have witnessed the association of norfloxacin resistant *V. cholerae* O1 and O139 causing cholera in contrast to the earlier years[10-11]. The combination of norfloxacin and ciprofloxacin resistant *V. cholerae* O1 and O139 were also encountered in each year of the study period. The MIC of ciprofloxacin, norfloxacin and nalidixic acid for the corresponding antibiotic resistant *V. cholerae* were found to be more than the susceptible values of each antibiotic in another study (Data of MIC values are not shown). Possibly, selective pressure exerted by nalidixic acid, disproportionate use of fluoroquinolones and increase in the incidence of nalidixic acid resistant strains of O1 and O139

(due to single mutation in *gyrA* and other related genes) could be the leading cause of appearance of fluoroquinolone resistance (with two or more mutation at *gyrA*). Mutant *V. cholerae* would have multiply and spread explosively, the progeny of which out number the fluoroquinolone susceptible Vibrios in the due course.

Tetracycline has been occupied as an important drug for control of cholera while chloramphenicol, furazolidone, co-trimoxazole and nalidixic acid are not less important. Over the time, tetracycline resistance has closely been monitored in various parts of the world. Reports of tetracycline resistant *V. cholerae* strains are appearing with increasing frequency[20]. Although tetracycline resistance in the present study was not high, however its emergence creates a great threat because the very presence of such resistance is an indication of the potential to spread. Almost all the strains of *V. cholerae* O1 and O139 were resistant to ampicillin, streptomycin and furazolidone, similar to the earlier studies[20] except increase of ampicillin susceptibility by O139 in 2000 and 2002. High incidence of co-trimoxazole resistant *V. cholerae* O1 in the present study is consistent with recent reports[20-21] while O139 strains revealed increased resistance in 2001 followed by 2002 in comparison to other years, reverse to the study conducted between 1992 to 1997 in Kolkata, India[20]. Chloramphenicol is the drug of choice during the scarcity of trimethoprim/sulphomethoxazole or resistance to it. However since one decade, chloramphenicol has been passing through several fluctuations in antibiogram pattern in world. With respect to this view, our study revealed moderate resistance to chloramphenicol by *V. cholerae* O1 and O139 strains.

Over the decades considerable enthusiasm has been developed for the study of multiple antibiotic resistances. The magnitude of this problem has been acknowledged since 1979 among *V. cholerae* O1[22-23] and unexpectedly increasing each year through different shifts[20, 24-25]. Unlike the antibiotic resistance profile AFz[26], AFzN[27] and AFzNS[28] carried by O139 vibrio and ACoFrNaS by O1[29] commonly encountered in Indian subcontinent and Bangladesh, different combination of multiple antibiotic resistance *V. cholerae* were recorded in this study. Analysis of drug resistance pattern during 1992-97 of clinical strains of *V. cholerae* in Calcutta[20], concluded that multiple antibiotic resistance pattern of *V. cholerae* O1 and O139 might be derived from the non-O1 and non-O139 strains during different time periods. If this were the case, it may be presumed that the resistance pattern carried by O1 and O139 in our study might be transferred from non-O1 and non-O139 and continued to exist for a particular period of time. However till date, the knowledge about the persistence period of resistance character in *V. cholerae* is poorly understood. In our study the evidence of CoFzNaS and ACoFzNaNSCNxCf antibiotic resistance profile carried by both the serogroup in a year, disappearance within a year after from O139 and persistence of the same profile intact with O1 hypothesizes that *V. cholerae* O1 can carry the drug resistance profiles for long period than O139 strains. To our knowledge, this is the first report on such incidence of multiple drug resistant profiles even to nine drugs among clinical strains of *V. cholerae* in India and the hypothesis to presume the period related occurrence of resistance character in individual strains of *V. cholerae.*

CONCLUSION

From the perspective of the detection of variable resistance pattern, the current emphasis is to address the dependence on antibiotic resistance test in time interval. If this approach is successful, then it will eventually become possible to determine which of them are effective antibiotics should be used. This capability represents an elegant approach in the control of cholera despite which the patients will be within the trap of disease and its consequences. Further advanced study is on progress to acknowledge the molecular mechanisms involved in the origin and spread of the antibiotic resistance of *V. cholerae*.

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REFERENCES

1. Carpenter, C.C.J. The treatment of cholera clinical science at the bedside. *J. Infect. Dis.*, **166**: 2-14 (1992).
2. Ghosh, S., Sengupt, P.G., Gupta, D.N. and Sirkar, B.K. Chemoprophylaxis studies in cholera; a review of selective works. *J. Commun. Dis.*, **24**: 5-57 (1992).
3. Lindenbaum, J., Greenough, W.B. and Islam, M.R. Antibiotic therapy of cholera. *Bull. World Health Organization.*, **166**: 871-883 (1967).
4. World Health Organization. The treatment of diarrhoea: a manual for physicians and other senior health workers, 4th revn, Geneva. *World Health Organization.*, (2005).
5. Thanassi, D.G., Cheng, L.W. and Nikaido, H. Active efflux of bile salts by *Escherchia coli. J. Bacteriol.*, **179**: 2512-2518 (1997).
6. Rouch, D.A., Cram, D.C., Diberadino, D., Little, S., Jhon, T.G. and Skurray, R.A. Efflux-mediated antiseptic resistance gene *qacA* from *staphylococcus aureus*: common ancestry with tetracycline and sugar transport proteins. *Mol. Mirobiol.*, **I4**: 2051-2062 (1990).
7. Ries, A.A., Wells, J.G., Olivola, D., Ntakibirora, M., Nyandwi, S., Ntibakivayo, M., Ivey, C.B., Greene, K.D., Tenevor, F.C., Wachlquist, S.P., Griffin, P.M. and Tauxe, R.V. Epidemic *Shigella* dysentery type-1 in Burundi: persistence and implication for prevention. *J. Infect. Dis.*, **169**: 1035-1041 (1994).
8. Threlfall, E.J., Ward, L.R., Rowe, B., Raghupathi, S., Chandrasekaran, V., Vandepitte, J. and Lemmens, P. Wide spread occurrence of multiple–drug resistance *Salmonella typhi* in India. *Eur. J. Clin. Microbiol. Infect. Dis.*, **11**: 990-993 (1992).
9. Weber, J.T., Mintz, E.D., Canizares, R., Semiglia, A., Gomez, I. and Sempertegui, R. Epidemic cholera in Ecuador: multidrug resistance and transmission by water and seafood. *Epidemiol. Infect.*, **112**: 1-11 (1994).
10. Pal, B.B., Khuntia, H.K., Anuradha, M. and Chhotray, G.P. Emergence of *V. cholerae* O139 during 1995 in Orissa, India- a retrospective study. *Ind. J. Med. Microbiol.*, **18**(4): 195-196 (2000).
11. Chhotray, G.P., Pal, B.B., Khuntia, H.K., Chowdhury, N.R., Chakraborty, S., Yamasaki, S., Ramamurthy, T., Takeda, Y., Bhattacharya, S.K. and Nair, G.B. Incidence and molecular analysis of *V. cholerae* associated with cholera outbreak subsequent to the super cyclone in Orissa, India. *Epidemiol. Infect.*, **128**: 131-138 (2002).
12. Kaper, J.B. Isolation, ecology and taxonomy of human pathogens in an estuary. Ph.D. Thesis, University of Maryland, College Park, M.D., U.S.A., (1979).
13. Garg, S., Ramamurthy, T., Mukhopadhyaya, A.K., Deb, B.C., Nair, G.B., Shimada, T., Takeda, T., Huq, A., Colwell, R.R. and Takeda, Y. Production and cross reactivity patterns of a panel of high affinity monoclonal antibodies to *V. cholerae* O139 Bengal. *FEMS. Immunol. Med. Microbiol.*, **8**: 293-298 (1994).
14. Bauer, A.W., Kirby, W.M.N., Sherri, J.C. and Turek, M. Antibiotic susceptibility testing by a standardized single disk method. *Amer. J. Clin. Path.*, **45**: 493-496 (1966).
15. World Health Organization. Epidemic diarrhoea due to *V. cholerae* non O1. *Wkl. Epidemiol. Rec.*, **68**: 141-142 (1993).
16. Hedges, R.W., Vialard, J.L., Pearson, N.J., Grady, F.O. Plasmids from Asian strains of *Vibrio cholerae*. *Antimicrob. Agents. Chemother.*, 11: 585-588 (1977).
17. Mukhopadhyay, A.M., Basu, I., Bhattacharya, S.K., Bhattacharya, M.K. and Nair, G.B. Emergence of fluoroquinolone resistance in of *V. cholerae* isolated from hospitalized patients with acute diarrhoea in Calcutta, India. *Antimicrob. Agents. Chemother.*, **42**: 206-207 (1998).
18. Krishna, B., Patil, A.B. and Chandrasekhar, M.R. Fluoroquinolone- resistant *V. cholerae* isolated during a cholera outbreak in India. *Trans. R. Soc. Trop. Med. Hyg.*, **100**(3): 224-226 (2006).
19. Bhattacharya, M.K., Ghosh, A.K.,

Mukhopadhyay, A.K., Deb, A. and Bhattacharya, S.K. Outbreak of cholera caused *V. cholerae* O1 intermediately resistant to norfloxacin at Malda, West Bengal. *J. Ind. Assoc.*, **98**(7): 389-390 (2000).

20. Garg, P., Chakraborty, S., Basu, I., Dutta, S., Rajendran, K., Bhattacharya, T., Yamasaki, S., Bhattacharya, S.K., Takeda, Y., Nair, G.B. and Ramamurthy, T. Expanding multiple antibiotic resistances among clinical strains of *V. cholerae* isolated from 1992-7 in Calcutta, India. *Epidemiol. Infect.*, **124**(3): 3932-3939 (2000).
21. Kaistha, N., Mehta, M., Gautam, V. and Gupta, V. Outbreak of cholera in around Chandigarh during two successive years (2002-2003). *Ind. J. Med. Res.*, **122**: 404-407 (2005).
22. Glass, R.I., Huq, I., Alim, A.R.M.A. and Yunus, M. Emergence multiple antibiotic-resistant *V. cholerae* in Bangladesh. *J. Infect. Dis.*, **142**: 939-942 (1980).
23. Glass, R.I., Huq, M.I., Lee, J.V., Threlfall, E.J., Khan, M.R., Alim, A.R.M.A., Rowe, B. and Gross, R.J. Plasmid-borne multiple drug resistance in *V. cholerae* serogroup O1, biotype El Tor: evidence of a point source outbreak in Bangladesh. *J. Infect. Dis.*, **147**: 204-209 (1983).
24. Tabtieng, R., Wattanasri, S., Echevearria, P., Seriwatana, J., Bodhidata, A., Chatkaeomorakot, A. and Row, B. An epidemic of *V. cholerae* El Tor Inaba resistant several antibiotics with a conjugative group C plasmid coding for type II dihydrofolate reduce in Thiland. *Am. J. Trop. Med. Hyg.*, **41**: 680-686 (1989).
25. Dalsgaard, A., Forslund, A., Petersen, A., Brown, D.J., Dias, F., Monterio, S., Molback, K., Aaby, P., Rodrigues, A. and Standstorm, D. Class I integron borne, multiple antibiotic resistance encoded by a 150-kilobase conjugative plasmid in epidemic *Vibrio cholerae* O1 strains isolated in Guinea-Bissau. *J. Clin. Microbiol.*, **38**: 3774-3779 (2000).
26. Faruque, S.M., Saha, M.N., Asadulghani, T., Bag, P., Bhadra, R.K., Bhattacharya, S.K., Sack, R.B., Takeda, Y. and Nair, G.B. Genomic diversity among *Vibrio cholerae* O139 strains isolated in Bangladesh and India between 1992 and 1998. *FEMS. Microbiol. Lett.*, **184**: 279-284 (2000).
27. Basu, A., Mukhopadhya, A.K., Sharma, C., Jyot, J., Gupta, N., Ghosh, A., Bhattacharya, S.K., Takeda, Y., Faruque, A.S.G., Albert, M.J. and Nair, G.B. Heterogeneity in the organization of CTX genetic element in strains of *Vibrio cholerae* O139 Bengal isolated from Calcutta, India and Dhaka, Bangladesh and its possible like to the dissimilar incidence of O139 cholera in the two locals. *Microbial. Pathogen.*, **24**: 175-183 (1998).
28. Mukhopadhyay, A.K., Basu, A., Garg, P., Bag, P.K., Ghosh, A., Bhattacharya, S.K., Takeda, Y. and Nair, G.B. Molecular epidemiology of re-emergent *Vibrio cholerae* O139, Bengal, India. *J. Clin. Microbiol.*, **36**: 2149-2152 (1998).
29. Bag, P.K., Maiti, S., Sharma, C., Ghosh, A., Basu, A., Mitra, R., Bhattacharya, S.K., Nakamura, S., Yamasaki, S., Takeda, Y. and Nair, G.B. Rapid spread of the new clone of the *V. cholerae* O1 biotype El Tor in cholera endemic areas in India. *Epidemiol. Infect.*, **121**: 245-251 (1998).