

## A Four Year Study Of Vibrio Cholerae Isolates At A Tertiary Care Centre

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**Abstract:** Background and objective: Cholera is an acute diarrhoeal disease caused by Vibrio cholerae. Cholera continues to be a major concern for the developing countries as it is an important cause of morbidity and mortality. Vibrio cholerae O1 El Tor Ogawa has been the offending agent since 1964. The present study was undertaken to determine prevalence, distribution of biotypes, serotypes, antibiotic susceptibility pattern of Vibrio cholerae and their MIC to tetracycline. Methods: Stool samples from suspected cases of cholera admitted were received in the laboratory for culture and sensitivity testing. The culture of specimens, identification and biochemical characterization of isolates were done as per standard techniques. Results: During the study period a total 450 stool samples were processed and 69 (15.33%) V.cholerae were isolated from them. All were El Tor O1 Ogawa. MIC of isolates also increased significantly during the study period. Interpretation and conclusion: Cholera is an acute type of diarrheal illness that affected millions of people around the world over the centuries. All the isolates (15.33%) were V.cholerae El Tor O1 Ogawa. Maximum isolation was in 2014 (36.23%). Resistance to tetracycline has increased from 6.25% in 2012 to 80% in 2015. Similarly resistance to ampicillin and cotrimoxazole has also increased substantially. Improved surveillance is useful in assessing the actual burden of cholera and in planning interventions. Increasing resistance pattern of V.cholerae is a matter of concern. [Kirti G NJIRM 2017; 8(1): 38-40]

**Key words :** Vibrio cholerae, El Tor, O1 Ogawa, MIC

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**Introduction:** Cholera is an acute diarrhoeal disease caused by Vibrio cholerae<sup>1</sup>. Cholera continues to be a major concern for the developing countries as it is an important cause of morbidity and mortality. It is an endemic disease with epidemics occurring at regular intervals<sup>2</sup>. The factors that predispose to epidemics are poor sanitation, malnutrition, overcrowding and inadequate medical services<sup>3</sup>. Vibrio cholerae O1 El Tor Ogawa has been the offending agent since 1964<sup>2</sup>. El Tor Vibrio cholerae have replaced their classical counterpart over the last few decades. Many recent reports of cholera outbreak in various parts of Indian subcontinent have been due to El Tor V.cholerae<sup>4</sup>

The use of antimicrobial agents is generally accepted as a method of reducing the duration and volume of diarrhoea as well as decreasing the period of V.cholerae excretion in stool<sup>5</sup>. The antimicrobial susceptibility pattern of V.cholerae O1 strains from newly infected patients are on constant change following the recent emergence and spread of multidrug resistant strains<sup>6</sup>. Therefore, the present study was undertaken to determine prevalence, distribution of biotypes, serotypes, antibiotic susceptibility pattern of Vibrio cholerae and their MIC to tetracycline

**Methods:** The study was carried out in Department of Microbiology, Indira Gandhi Government Medical College and Hospital, Nagpur from January 2012 to August 2015. Stool samples from suspected cases of

cholera admitted were received in the laboratory for culture and sensitivity testing. The culture of specimens, identification and biochemical characterization of isolates were done as per standard techniques<sup>7</sup>. Biotyping of isolates was done by Voges-proskauer test and polymyxin B susceptibility. Serotyping of the strains was performed by using V. cholerae high titre antisera against O1, Ogawa and Inaba received from Central Research Institute Kasauli and O139 obtained from National Institute of Cholera and Enteric Diseases (NICED), Kolkata. Antimicrobial susceptibility testing was done using the Kirby-Bauer disc diffusion method as per CLSI guidelines<sup>8</sup>. The antibiotic disks tested were tetracycline (30µg), cotrimoxazole (25µg), ampicillin (10µg) and chloramphenicol (30µg). The minimum inhibitory concentration (MIC) of tetracycline was determined by the agar dilution method.

**Results:** During the study period a total 450 stool samples were processed and 69 (15.33%) V.cholerae were isolated from them. All were El Tor O1 Ogawa. Maximum isolates were found in year 2014 (Table I). During the study period V.cholerae seems to have gained resistance to antibiotics. In 2012 resistance to ampicillin and chloramphenicol was almost nil but over the years it increased to 60% and 50% respectively. Resistance to tetracycline also increased substantially (Table II). MIC of isolates also increased significantly during the study period (Table III).

**Table I : Year wise distribution of Vibrio cholerae isolates**

Year	Total samples	Vibrio isolates
2012	75	16 (23.19)
2013	133	18 (26.09)
2014	190	25 (36.23)
2015	52	10 (14.49)
Total	450	69 (15.33)

**Table II : Antibiotic resistance pattern of isolates during the study period**

Antibiotic	2012 (n=16)	2013 (n=18)	2014 (n=25)	2015 (n=10)	Total (n=69)
Ampicillin	0 (0)	1 (5.55)	3 (12)	6 (60)	10 (14.49)
Tetracycline	1(6.25)	2 (11.11)	6 (24)	8 (80)	17 (24.63%)
Cotrimoxazole	1 (6.25)	1 (5.55)	5 (20.8)	6 (60)	13 (18.84%)
Chloramphenicol	0 (0)	1 (5.55)	4 (16)	5 (50)	10 (12.49)

**Table III: MIC of V.cholerae to tetracycline**

Year	MIC of Tetracycline ( $\mu\text{g/ml}$ )					Total
	2	4	8	16	32	
2012	5 (31.3%)	10(62.5%)	0	1(6.2%)	0 (0)	16
2013	5 (27.8%)	11(61.1%)	0	2(11.1%)	0 (0)	18
2014	5 (20.0%)	14 (56.0%)	0	5(20.0%)	1 (4.0%)	25
2015	0 (0)	2 (20.0%)	0	4(40.0%)	4(40.0%)	10
Total	15(21.7%)	37(53.6%)	0	12(17.4%)	5 (7.3%)	69

Sensitive -  $\leq 4 \mu\text{g/ml}$ , Intermediate-  $8 \mu\text{g/ml}$ , Resistant-  $\geq 16 \mu\text{g/ml}$

**Discussion:** Cholera is an acute type of diarrheal illness that affected millions of people around the world over the centuries. Historically speaking, there are very few diseases that can match cholera in terms of its severity and explosive onset in the form of an outbreak or epidemic<sup>9</sup>.

The present study gives a review of the cholera scenario in our region from 2012 to 2015. During the study period average isolation of V.cholerae from suspected stool samples received was 15.33%. All the isolates were V.cholerae El Tor O1 Ogawa. Maximum isolation was in 2014 (36.23%). In 2014, our region witnessed an outbreak of cholera and this high isolation is attributable to that outbreak. The genome of V.cholerae is in a state of constant change, resulting in the emergence of isolates that can either initiate an outbreak or are capable of perpetuating their species in spite of existing high immunity in the community. In the era of rapid transport, there is every possibility of the new strain

finding its way into new population and initiating an outbreak<sup>10</sup>. Annual outbreaks of cholera are a regular

feature in our country. A high population density along with open drains and poor sanitation provides an optimal niche for survival, sustenance and transmission of V.cholerae<sup>5</sup>.

The study has also shown that the isolates in our laboratory had an increasing tendency to develop resistance to routinely used antibiotics. This resistance has been observed to all the recommended antibiotics. Resistance to tetracycline has increased from 6.25% in 2012 to 80% in 2015. Similarly resistance to ampicillin and cotrimoxazole has also increased substantially. This pattern of quick shift in resistance is consistent with reports from other researchers and it indicates an enhanced mobility in genetic elements like transposons and integrons which confer resistance to antibiotics<sup>3,10</sup>. Since the treatment of severe cases of diarrhoea is important, the emergence of resistance of V.cholerae to the most important agents of therapy for cholera is a matter of concern.

**Conclusion:** our study shows that El Tor and Ogawa are the prevalent biotypes and serotypes of V.cholerae in our region. Improved surveillance is

useful in assessing the actual burden of cholera and in planning interventions. Increasing resistance pattern of *V.cholerae* is a matter of concern. There is need for control interventions in high risk areas including vaccines, along with improved access to safe water and adequate sanitation.

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