

## Resistotypes of *Vibrio cholerae* 01 Ogawa Biotype El Tor in Kathmandu, Nepal

R Karki,<sup>1</sup> DR Bhatta,<sup>1</sup> S Malla,<sup>2</sup> SP Dumre,<sup>2,3</sup> BP Upadhyay,<sup>2</sup> S Dahal<sup>1</sup> and D Acharya<sup>1</sup>

<sup>1</sup>Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal, <sup>2</sup>National Public Health Laboratory, Kathmandu, Nepal, <sup>3</sup>Faculty of Allied Health Sciences, Thammasat University, Thailand

**Corresponding author:** Shyam Prakash Dumre, Graduate Program in Biomedical Sciences, Faculty of Allied, Health Sciences, Thammasat University (Rangsit Campus), Klongluang, Pathumthani, 12121, Thailand; e-mail: shyamprad@hotmail.com

### ABSTRACT

*Cholera continued to be a major diarrheal illness in Nepal and antibiotic resistance has appeared as a serious problem in cholera management. The study aimed at analyzing the distribution pattern of the resistotypes (R-types) of Vibrio cholerae in the Kathmandu valley, Nepal. During June 2008 to January 2009, 210 diarrheal specimens received at National Public Health Laboratory from suspected cholera patients were subjected to standard bacteriological investigation including biotyping and serotyping. Antimicrobial susceptibility pattern of V. cholerae isolates was determined by Kirby Bauer disc diffusion method following CLSI guidelines. A total of 57 (27%) V. cholerae isolated were recovered, all of which belonged to 01 Ogawa Biotype EL Tor. Based on antibiogram, V. cholerae isolates in our study revealed three distinct R-types: R-type I, R-type II and R-type III. All three R types showed resistance to furazolidone, nalidixic acid and cotrimoxazole while sensitive to ciprofloxacin and tetracycline. Additional resistance to ampicillin and erythromycin was observed respectively in R-type II and III. Different R-types showed unique month wise variations (P<0.05). Differentiation of V. cholerae strains into R-types is an important tool. In addition to direct patient management, it may have implication in identifying the source and spread of infection, and understanding the distribution pattern in a particular geographical region.*

**Keywords:** Cholera, Vibrio, R-types, Nepal.

### INTRODUCTION

Cholera, a severe watery diarrheal disease, is caused by cholera toxin (CT) producing strains of *V. cholerae* which colonizes the small intestine.<sup>1,2</sup> In its most severe form, there is rapid loss of fluid and electrolytes from the gastrointestinal tract resulting in hypovolemic shock, metabolic acidosis, and if untreated death.<sup>1-4</sup> *V. cholerae* strains causing cholera epidemics have been confined mainly to the CT-producing serogroup O1 and O139, except the occasional report of non-O1 non-O139 serogroups.<sup>5</sup> Despite the improved effort in health awareness and sanitation, cholera outbreak is common in Nepal since years.<sup>6-10</sup> Recent massive cholera outbreak was observed in the mid-western region of Nepal. During May 2009, there were around 5000 cases and over 200 deaths due to diarrhea in the western region of Nepal, and this outbreak was substantially incriminated to cholera.<sup>11</sup> In Nepal bacteriological quality of drinking water may becomes below standard particularly during rainy season through contamination with fecal matters<sup>12,13</sup> contributing outbreaks of diarrhea and other gastrointestinal tract infections.

The primary treatment for cholera is rehydration, however antibiotic therapy is the mainstay in shortening the course of infection, preventing person-to-person

transmission and protecting immunocompromised individuals victimized by cholera.<sup>14</sup> Although tetracycline and doxycycline are the recommended drugs in cholera management, other antibiotics like furazolidone, erythromycin, cotrimoxazole, chloramphenicol and ciprofloxacin are effective alternatives when susceptible.<sup>15,16</sup>

Periodic monitoring for R-types could be crucial in cholera endemic regions since *V. cholerae* has expanded its resistance capabilities or R-types.<sup>17</sup> Due to the progressive increase in antimicrobial resistance in *V. cholerae* and existence of changing resistance pattern, differentiation of *V. cholerae* strains into R-types is of clinical, public health and epidemiological importance. Currently, there is no adequate information on R-types of *V. cholerae* in context of Nepal. This study aimed to identify the different R-types of *V. cholerae*, and analyze their distribution pattern at the national reference laboratory of Nepal.

### MATERIALS AND METHODS

**Study design:** Exploratory study design was adopted in order to find out the period prevalence and distribution of different R-types of *V. cholerae*. Between June 2008 to January 2009, 210 stool samples from suspected

**Table-1:** Distribution of R-types of *V. cholerae* 01 Ogawa Biotype EL Tor

R Types	Antibiotic resistance Pattern**	Months				Total*
		Jun	Jul	Aug	Sept	
I	FR-NA-Co	8	8	5	4	25(44)
II	FR-NA-Co-Amp	0	7	7	0	14(24)
III	FR-NA-Co-E	0	1	1	16	18(32)
	Total*	8(14)	16(28)	13(23)	20(35)	57(100)

\*Figures in the parenthesis indicate percentage, \*\*Abbreviations used are described in Fig. 1.

cholera patients were included in the study and subjected to bacteriological investigation at National Public Health Laboratory (NPHL), Kathmandu, Nepal. The time frame of the study was selected on the basis of seasonal trend of cholera in Nepal reported previously.<sup>6,7,10</sup>

**Isolation and identification of *V. cholerae*:** Stool specimens were processed following standard microbiological procedures and *V. cholerae* was isolated and identified by colony morphology, Gram's staining and biochemical tests<sup>18</sup> and further analyzed by serotyping using specific anti-sera (Denka Seiken Co. Ltd, Japan). Resistance to polymyxin, positive Voges-proskauer reaction and hemolysis of sheep erythrocytes were used as criteria for distinguishing El Tor from classical biotype of *V. cholerae* 01.<sup>19</sup>

**Antimicrobial susceptibility testing:** *V. cholerae* isolates were tested for susceptibility to antibiotics by Kirby-Bauer disc diffusion method using commercially available discs (Mast, UK). The following antibiotics were used: ampicillin (10 µg), tetracycline (30 µg), erythromycin (15 µg), ciprofloxacin (5 µg), nalidixic

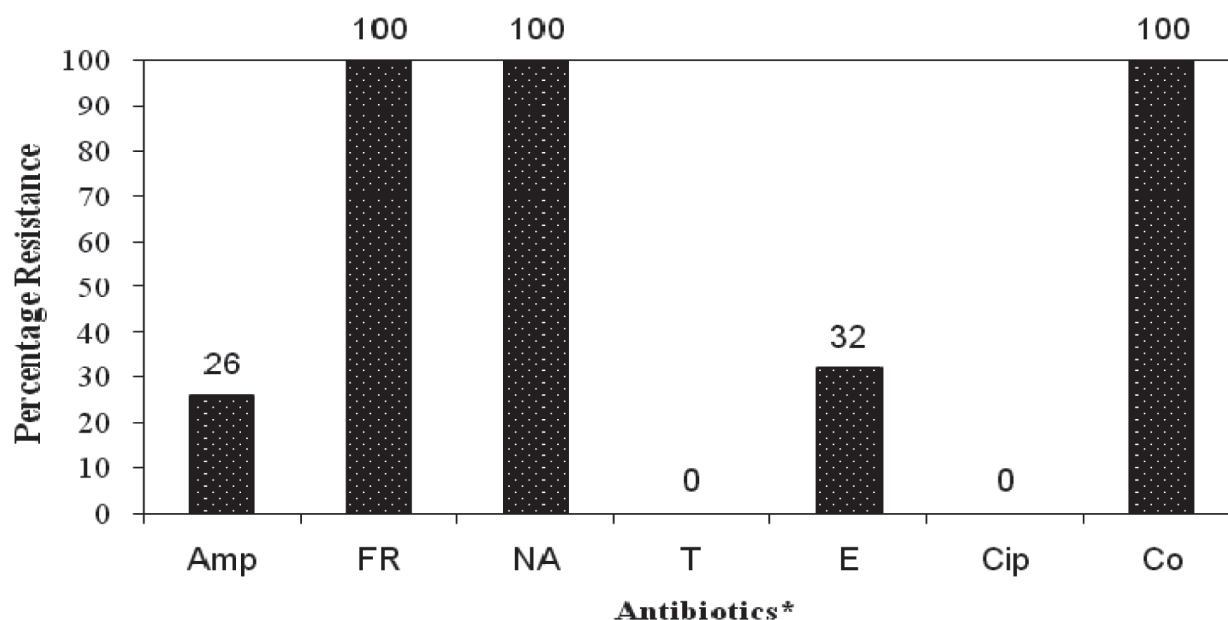
acid (30 µg), cotrimoxazole (25 µg) and furazolidone (100 µg). Isolates were characterized as susceptible and resistant based on diameter size of the zone of inhibition (ZOI) according to the Clinical and Laboratory Standards Institute (CLSI). Any isolates with intermediate ZOI were interpreted as being resistant to that drug.

**Data analysis:** Finally, the isolates were classified into R-types based on their resistance patterns. Chi-square test was applied to determine significant differences.

## RESULTS

Out of 210 stool samples, 57 (27%) *V. cholerae* were isolated and all isolates were 01 Ogawa Biotype El Tor. Interestingly, all the isolates of *V. cholerae* were recovered from the stool samples collected during the period of June to September.

Furazolidone, nalidixic acid and cotrimoxazole were found to be 100% resistant while none of the isolates were found resistant against tetracycline and ciprofloxacin (Fig. 1). Based on their antibiogram, three distinct R-types (R-type I, II and III) of *V. cholerae* 01



**Fig. 1.** Antimicrobial resistance pattern of the *V. cholerae* 01 isolates

\*Amp: ampicillin, FR: furazolidone, NA: nalidixic acid, Co: cotrimoxazole, Cip: ciprofloxacin, T: tetracycline, E: erythromycin

Ogawa Biotype El Tor were observed (Table-1). All three R-types were resistant to furazolidone, nalidixic acid, and cotrimoxazole. Additional resistance to ampicillin and erythromycin was observed respectively in R-type II and R-type III. However, all three R-types were sensitive to ciprofloxacin and tetracycline.

Month wise distribution of different R-types was not uniform throughout the study period (Table 1). R-type I (44%) pre-dominated R-Type II (24%) and R-type III (32%) with statistical significance ( $P < 0.05$ ). R-type I was common during all the *V. cholerae* reported months. R-type II appeared during July and persists till August, while R-type III though appeared lately in July, predominated rest two types significantly ( $P < 0.05$ ) in the month of September. Moreover, R-type I was the only type recovered during the month of June.

## **DISCUSSION**

All *V. cholerae* isolates in this study belonged to serogroup O1 Ogawa biotype El Tor. However, all the isolates previously reported in this reference laboratory during 2003-2004 were *V. cholerae* O1 Ogawa biotype El Tor and there was a complete serotype conversion to Inaba (100%) in 2005 and 2006. Interestingly, in 2007, all three serotypes: Ogawa (64%), Inaba (35%) and Hikojima (1%) were reported.<sup>20</sup> Serotype Hikojima was reported earlier in 1996 from Kathmandu<sup>6</sup> but never exceeded to a large number in the successive years. *V. cholerae* O1 El Tor Ogawa was the major serotypes reported from Nepal in the past years, although serotype conversion from Ogawa to Inaba in some years and settings is common which couldn't be undermined. Such a serotype shifting is a common phenomenon in *V. cholerae* and was also reported from India<sup>1,21,22</sup>, a country that borders Nepal.

All the 57 isolates showed resistance to cotrimoxazole, nalidixic acid and furazolidine. Additional resistance to erythromycin or ampicillin was also observed in some isolates. The high prevalence of multidrug resistance (MDR) in *V. cholerae* has been a matter of concern as it limits the therapeutic alternatives. Although all isolates were uniformly susceptible to tetracycline and ciprofloxacin, the former one is not recommended for use in children while the latter is also not advocated during pregnancy and childhood. Besides this, there are reservations about the use of ciprofloxacin in treating cholera caused by nalidixic acid resistant strains<sup>23</sup> and possibility of the emergence of ciprofloxacin resistance in *V. cholerae*.<sup>24</sup> The association between nalidixic acid resistance and reduced susceptibility to ciprofloxacin is also documented in other enteric pathogens from Nepal.<sup>25</sup>

Typing of *V. cholerae* into different R-Types is an important epidemiological marker and has already been practiced in some settings.<sup>26</sup> We found that three different R-types of *V. cholerae* O1 Ogawa Biotype EL Tor were prevalent in Kathmandu valley. While considering the month-wise distribution patterns of *V. cholerae* R-types, R-type II was present during the months of July and August only, whereas majority of R-type III was recovered during September and R-type I was common throughout the cholera reported months. There was significant variation in the distribution pattern ( $p < 0.05$ ) of these three R-types by month. It implies that there might be different factors to play role in cholera transmission, and more importantly in development of different antibiotic resistance patterns. This information could be considered in clinical as well as epidemiological implications.

Variation in the month wise distribution of R-types indicates that there might be some internal factors or genes that cause to engender optimum milieu for one R-type than the rest. This assumption also includes the possibility that the same antibiotic resistance gene(s) that contributed an isolate to become of particular R-type may favor it to prosper in contrast to its counterpart R-types deficient in that particular gene(s). Analyzing the roles of various environmental and genetic factors in the month wise distribution of the R-types might be a further research perspective.

Despite the improvement in health awareness and sanitation, cholera outbreaks are still common in Nepal with considerable number of MDR *V. cholerae*.<sup>7,8,9,27</sup> Although most cholera patients will recover if they are rehydrated promptly and properly, antibiotics may be crucial particularly in lowering the volume of excreted fluid via stool, reducing the illness period and vibrios excretion in feces.<sup>28</sup> More importantly, antibiotic therapy helps in slowing down the outbreaks because organisms from infected persons may be more virulent than those acquired in the wild type.<sup>14</sup> It is an utmost importance to know exactly which R-type of *V. cholerae* is currently circulating in a particular geographical location, so that effective antibiotic could be administered for clinical management. As most of the commonly used antibiotics in our settings are not working at all in vitro except ciprofloxacin and tetracycline, this information might have implications in formulating empirical treatment policy for cholera. As the results are based on the exploratory observations made at a national reference laboratory which may not necessarily represent the actual national scenario of *V. cholerae* and its R-type distribution. This emphasizes on the need of continuous surveillance in the country.

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