

Short Communication

Cholera Incidence among Patients with Diarrhea Visiting National Public Health Laboratory, Nepal

Rabindra Karki*, Dwij Raj Bhatta, Sarala Malla¹, and Shyam Prakash Dumre¹

Central Department of Microbiology, Tribhuvan University, Kirtipur; and ¹National Public Health Laboratory, Kathmandu, Nepal

(Received October 14, 2009. Accepted March 11, 2010)

SUMMARY: The major objective of this study was to deliver vital statistics related to cholera to health authorities so as to aid in their attempt to prioritize communicable diseases in Nepal. A laboratory-based surveillance was conducted from mid-June 2008 to mid-January 2009 at the National Public Health Laboratory, Nepal. Diarrheal samples alone were processed for *Vibrio cholerae*. Isolation and identification of the organisms were carried out as per standard protocol. Antimicrobial susceptibility tests were done according to the guidelines of the Clinical and Laboratory Standards Institute. The incidence of cholera was found to be 27.1%. Only *V. cholerae* O1 Ogawa biotype El Tor was found during the study. No variation was observed in the percentage of cases between genders ($P < 0.05$). The 15–30 year age group was found to be more susceptible to cholera ($P < 0.05$). The period from mid-June to mid-July had the highest incidence of cholera ($P < 0.05$). Ampicillin, tetracycline, ciprofloxacin, and erythromycin were highly effective, while 100% resistance was observed for furazolidone, nalidixic acid, and cotrimoxazole.

According to the World Health Organization, an estimated 120,000 deaths from cholera occur globally every year (1). A yearly mortality of at least 30,000 and morbidity of 3.3 episodes per child was estimated due to diarrhea in Nepal (2). One of the most important causes of acute diarrhea in Nepal is cholera (3).

Vibrio cholerae, the causative agent of cholera is erratic in its nature and nobody knows when a non-toxicogenic strain of this pathogen may be empowered with virulence properties possibly causing havoc or a pandemic throughout the world, like the present day El Tor *Vibrio* (4). A constant vigilance of this etiological agent is of paramount importance. Literature regarding incident rates of cholera in Nepal are rare, and hence this study is valuable for health authorities involved in prioritizing communicable diseases.

A laboratory-based surveillance was conducted from mid-June 2008 to mid-January 2009 at the National Public Health Laboratory, Nepal. A total of 210 diarrheal samples were processed during the study period. *V. cholerae* was isolated and identified by standard laboratory methods (5,6) and confirmed by serotyping using specific antisera (Denka Seiken, Tokyo, Japan). Resistance to polymyxin B (300 units), positive Voges-Proskauer reaction and hemolysis of sheep erythrocytes were used as criteria for distinguishing El Tor from the classical biotype of *V. cholerae* O1 (7–10). Isolates were tested for susceptibility to antibiotics by the Bauer-Kirby disc diffusion method using commercially available discs (Hi-Media, Mumbai, India). The following antibiotics were used: ampicillin (10 µg), tetracycline (30 µg), erythromycin (15 µg), ciprofloxacin (5 µg), nalidix-

ic acid (30 µg), cotrimoxazole (25 µg), and furazolidone (100 µg). Isolates were characterized as susceptible, intermediate, or resistant based on the size of the inhibition zones in accordance with the manufacturer’s guidelines.

The chi-square test was applied to determine significant differences. A susceptibility percentage for each antimicrobial tested was calculated by dividing the number of susceptible isolates by the total number of tested isolates.

Altogether 210 diarrheal samples were processed for *V. cholerae*. Out of these samples only 57 were positive for *V. cholerae* yielding an incidence of 27.1%. No significant difference of cholera incidence was seen between males (28 cases) and females (29 cases) ($P < 0.05$). The 15–30 year age group had the highest number of cholera cases ($P < 0.05$) (Fig. 1). The period from mid-June to mid-July had the highest incidence of

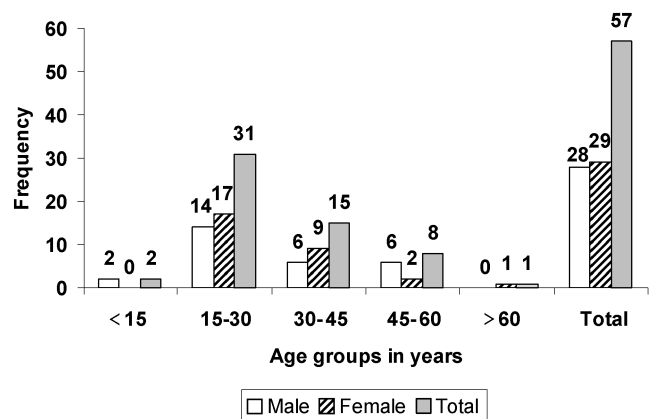


Fig. 1. Age-wise distribution of cholera cases.

*Corresponding author: Mailing address: Central Department of Microbiology, Tribhuvan University, Kirtipur, Nepal. Tel: +977-9841173578, E-mail: Ki2rabi2009@yahoo.com

Table 1. Antimicrobial susceptibility patterns of the isolates

Antibiotic	Sensitive	Intermediate	Resistant
	No. (%)	No. (%)	No. (%)
Ampicillin	42 (73.6)	5 (8.7)	10 (17.5)
Furazolidone	0 (0)	0 (0)	57 (100)
Nalidixic acid	0 (0)	0 (0)	57 (100)
Cotrimoxazole	0 (0)	0 (0)	57 (100)
Ciprofloxacin	57 (100)	0 (0)	0 (0)
Tetracycline	57 (100)	0 (0)	0 (0)
Erythromycin	39 (68.4)	18 (31.5)	0 (0)

cholera ($P < 0.05$). Antimicrobial susceptibility testing of the isolates showed ampicillin, tetracycline, erythromycin, and ciprofloxacin to be effective whereas isolates were found to be 100% resistant to furazolidone, nalidixic acid, and cotrimoxazole (Table 1).

In this study, all 57 isolates belonged to *V. cholerae* O1 and no *V. cholerae* O139 were isolated. This is consistent with the findings of Kubo and Pokhrel (11), Yamamoto et al. (12), and Ono et al. (13), all of whom were unable to detect *V. cholerae* O139.

All of the *V. cholerae* O1 belonged to serotype Ogawa and biotype El Tor. Yamamoto et al. (12) also reported isolation of only *V. cholerae* O1 biotype El Tor. Ise et al. (14) also reported isolation of *V. cholerae* O1 biotype El Tor, Ogawa from the capital only. Kaistha et al. (15) also reported isolation of *V. cholerae* O1 Ogawa biotype El Tor only during the year 2002 and 2003 in Chandigarh, India. Mandomando et al. (16) reported isolation of *V. cholerae* O1 Ogawa only during the period from November 2002 to April 2003 and from November 2003 to April 2004 in southern Mozambique.

However, in 1996, Kubo and Pokhrel (11) reported isolation of mixed serotypes of *V. cholerae* O1, viz. Hikojima and Ogawa, with a preponderance of Hikojima serotype from cholera cases in the capital city of Kathmandu. Also, the National Public Health Laboratory in Kathmandu has reported isolation of *V. cholerae* O1 Ogawa in 2004 and before 2004, Inaba strains of *V. cholerae* O1 during 2005 and 2006, and isolation of both Ogawa and Inaba during 2007 (unpublished data). In East Delhi, *V. cholerae* O1 Ogawa was the only predominant isolate during the period from 2001 to 2003. However, from 2004 to 2006 *V. cholerae* O1 Inaba predominated the coexisting Ogawa serotype (17). In Kolkata, India, *V. cholerae* O1 Ogawa serotype predominated the Inaba serotype during 2004, while the reverse was true for 2005 (18).

In this study no significant difference in the number of cholera cases between genders was seen ($P < 0.05$). The above finding is in concordance with that of Ono et al. (13), who reported no significant difference in the detection rate of enteropathogens between male and female populations.

As reported above, an age-wise distribution study showed that the most prevalent age group for cholera cases was from 15–30 years, with the next most prevalent the age group from 30–45 years, followed by 45 years and above ($P < 0.05$). The 15–30 year age group is highly productive and involved in various sorts of daily activities which may lead to increased exposure to

cholera agents and increased susceptibility. The decrease in cholera cases from 30–60 years may be due to a propensity of this age group toward a sedentary life style with a lower risk of exposure to etiological agents. Kaistha et al. (15) reported a high percentage of cholera infection in children during 2002 and 2003 in Chandigarh, India. However in this study, the fact that the age group below 15 is seen as the least vulnerable group may be a form of Berkson's bias, as the children in the age group below 15 years are usually taken to separate children's hospitals such as the Kanti Children Hospital, Nepal.

Outbreaks of cholera are a regular feature in Nepal. The seasonal outbreaks of cholera are a reminder of the endemic characteristic of the illness and its emergence as an important pathogen of acute watery diarrhea (17).

In Nepal, normally the dry season starts in the month of Chaitra (March/April) and continues until Jestha (May/June). The rainy season starts in Ashad (June/July) and continues until Aswin (September/October). Waterborne epidemics such as diarrhea, gastroenteritis, typhoid, and cholera occur in these seasons because of insufficient water, poor water quality, and unsanitary conditions. During the dry season, there is an acute scarcity of drinking water, while in the rainy season, although the quantity of water available is large, most water sources are contaminated with excreted microorganisms from surface water runoff (2).

In this study, which was conducted from June 2008 to January 2009, the incidence of cholera was found to be highest (57.14%) during Ashad (June/July) ($P < 0.05$). Ise et al. (14) also reported an outbreak of cholera in Kathmandu from July to September (rainy season) in 1994.

In this study the antimicrobial susceptibility patterns of the isolates revealed that the isolates were sensitive to ciprofloxacin, tetracycline, ampicillin, and erythromycin. However, the isolates were 100% resistant to furazolidone, cotrimoxazole, and nalidixic acid. The appearance of resistance to cotrimoxazole is a severe threat to a country such as Nepal, where it is widely used to treat acute gastroenteritis. *V. cholerae* O1 Ogawa biotype El Tor were isolated during 1994, and the isolates revealed high sensitivity to tetracycline, ciprofloxacin, and gentamycin and low sensitivity to nalidixic acid, cotrimoxazole, ampicillin, and cephalixin (14).

There is a wide array of mechanisms that lead to the development of resistance to antibiotics in bacteria. The emergence of multiple drug resistance is a serious problem in the treatment and containment of the disease as reflected by the increase in the fatality rate from 1 to 5.3% after the emergence of drug resistant strains in Guinea Bissau during the 1996–97 epidemic of cholera (15).

In conclusion, the high incidence rate of diarrhea due to cholera should not be ignored. The high degree of resistance observed appears to have resulted from the practice of indiscreet use of antibiotics in Nepal. Concrete action should be taken by the relevant authorities to prevent strains from developing resistance to the paucity of drugs that are effective now.

Acknowledgments The authors are grateful to the National Public Health Laboratory, Nepal, for providing such a conducive environment for this research work.

REFERENCES

1. Zhang, D., Xu, Z., Sun, W., et al. (2003): The *Vibrio* pathogenicity island-encoded mop protein modulates the pathogenesis and reagentogenicity of epidemic *Vibrio cholerae*. *Infect. Immun.*, 71, 510–515.
2. Pokhrel, D. and Viraraghavan, T. (2004): Diarrhoeal diseases in Nepal *vis-à-vis* water supply and sanitation status. *J. Water Health*, 2, 71–81.
3. World Health Organization (2007): Flood in Disaster: Nepal. Communicable Disease Risk Assessment and Interventions. August 2007. World Health Organization, Geneva.
4. World Health Organization (2009): Global Epidemics and Impact of Cholera. Online at <<http://www.who.int/topics/cholera/impact/en/index.html>>.
5. Forbes, B.A., Sahm, D.F. and Weissfeld, A.S. (2007): Bailey and Scott's Diagnostic Microbiology. 12th ed. Mosby.
6. Vandepitte, J., Verhaegen, J., Engbaek, K., et al. (2000): Stool. p. 37–59. Basic Laboratory Procedures in Clinical Bacteriology. 1st ed. A.I.T.B.S. Publishers and Distributors, Delhi.
7. Janda, J.M., Powers, C., Bryant, R.G., et al. (1988): Current perspectives on the epidemiology and pathogenesis of clinically significant *Vibrio* spp. *Clin. Microbiol. Rev.*, 1, 245–267.
8. Dziejman, M., Balon, E., Boyd, D., et al. (2002): Comparative genomic analysis of *Vibrio cholerae*: genes that correlate with cholera endemic and pandemic disease. *Proc. Natl. Acad. Sci. USA*, 99, 1556–1561.
9. Guidolin, A. and Manning, P.A. (1987): Genetics of *Vibrio cholerae* and its bacteriophages. *Microbiol. Rev.*, 51, 285–298.
10. Calia, K.E., Murtagh, M., Ferraro, M.J., et al. (1994): Comparison of *Vibrio cholerae* O139 with *V. cholerae* O1 classical and El Tor biotypes. *Infect. Immun.*, 62, 1504–1506.
11. Kubo, T. and Pokhrel, B.M. (1996): Outbreaks of cholera in Nepal. *Southeast Asian J. Trop. Med. Public Health*, 27, 574–579.
12. Yamamoto, K., Shrestha, J., Iida, T., et al. (1995): Molecular epidemiology of *Vibrio cholerae* O1 isolated in Nepal by Southern hybridization with a cholera toxin gene probe. *J. Diarrhoeal Dis. Res.*, 13, 113–117.
13. Ono, K., Rai, S.K., Chikahira, M., et al. (2001): Seasonal distribution of enteropathogens detected from diarrheal stool and water samples collected in Kathmandu, Nepal. *Southeast Asian J. Trop. Med. Public Health*, 32, 520–526.
14. Ise, T., Pokharel, B.M., Rawal, S., et al. (1996): Outbreaks of cholera in Kathmandu Valley in Nepal. *J. Trop. Pediatr.*, 42, 305–307.
15. Kaistha, N., Mehta, M., Gautam, V., et al. (2005): Outbreak of cholera in & around Chandigarh during two successive years (2002, 2003). *Indian J. Med. Res.*, 122, 404–407.
16. Mandomando, I., Espasa, M., Vallés, X., et al. (2007): Antimicrobial resistance of *Vibrio cholerae* O1 serotype Ogawa isolated in Manhiça District Hospital, southern Mozambique. *J. Antimicrob. Chemother.*, 60, 662–664.
17. Das, S., Saha, R. and Kaur, I.R. (2008): Trend of antibiotic resistance of *Vibrio cholerae* strains from East Delhi. *Indian J. Med. Res.*, 127, 478–482.
18. Roychowdhury, A., Pan, A., Dutta, D., et al. (2008): Emergence of tetracycline-resistant *Vibrio cholerae* O1 serotype Inaba, in Kolkata, India. *Jpn. J. Infect. Dis.*, 61, 128–129.