Study of medically important Vibrios in the sewage of Katmandu Valley, Nepal

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ABSTRACT

A total of 42 samples were collected from the different sites of sewer system of Kathmandu Valley during rainy summer season (June to September 2008) using Moore's technique. Samples (on Moore's swabs) were submersed in alkaline peptone water (pH 8.6) and transported to Research Laboratory of National Institute of Tropical Medicine and Public Health Research, Kathmandu in cold condition (ice chest) followed by incubation at 37^{0}C for 8 hours. After incubation, culture was done on thiosulfate-citrate-bile salt-sucrose (TCBS) agar and incubated at 37^{0}C for overnight (15 hrs). Both yellow and green colonies measuring from 2 to 9 mm in diameter on TCBS agar were subjected for gram staining, biochemical testing as well as sero-typing using anti-sera (poly O1, Ogawa and Inaba) (Denka Seiken Co. Ltd, Japan). Altogether 46 medically important *Vibrios* were isolated from 42 samples studied. The isolates were identified as *V. cholerae* (n=20; 43.5%), *V. vulnificus* (n=11; 23.9%), *V. parahaemolyticus* (n=5; 10.9%), *V. furnissi* (n=5; 10.9%), *V. fluvialis* (n=3; 6.5%) and *V. alginolyticus* (n=2; 4.3%). Of the 20 *V. cholerae* isolates, 13 (65.0%) and 7 (35.0%) isolates were *V. cholerae* O1 and non-O1, respectively. Among the *V. cholerae* O1 (n=13), classical Hikojima strain was most frequently isolated (n=10) followed by *V. cholerae* O1 Ogawa (El Tor=2 and classical=1). High frequency of *V. cholerae* isolation from sewer system of Kathmandu Valley is an indication of possible outbreak of cholera anytime in future and, therefore, demands improvement in sanitary condition, supply / consumption of safe drinking water and personal hygiene.

Key words: *V. cholerae*, sewer system, Kathmandu Valley, Nepal.

INTRODUCTION

Vibrio species are abundant in aquatic environment worldwide.[1-4] Of them, dozens of species are implicated in some kinds of illness in man. *Vibrios* frequently associated with human health include *V. cholerae*, *V. parahaemolyticus, V. fluvialis, V. vulnificus, V. furnissi* and others.[5] *V. cholerae* alone is responsible for high morbidity and mortality.[5] Cholera caused by toxigenic *V. cholerae* O1 and O139 Bengal strain outbreak is common in most of the developing countries.[6,7] According to recent WHO estimate, 3–5 millions cases and 100,000–120,000 deaths due to cholera occur every year.[8] Cholera cases and/or outbreaks are associated with poor environmental sanitation resulting into contamination of water and foods as well as lack of personal hygiene.

Gastroenteritis (associated with diarrhoea) is one of the major health problems in Nepal causing high morbidity (30,000 death/year) and mortality of 3.3 episodes per child.[9] First bacteriologically confirmed epidemic cholera (subject of an international report) from Nepal was reported in 1958.[10] There are many reports of the isolation of *V. cholerae* from the clinical samples including from cases of outbreaks.[11,12] In Nepal, outbreaks of cholera occur each year with the beginning of summer/rainy season (continues to post rainy season)[13-15] and mainly associated with *V. cholerae* 01 biotype El Tor Ogawa.[12,16] This is true even in the Katmandu Valley where the capital city is located.[13]

Bagmati River is the biggest river running across the Kathmandu Valley. This river also has religious importance especially for *Hindu* devotees. The devotees take holy bath in the river water and also drink the water particularly at *Pashupati Nath* temple (the famous god *Shiva* temple) area. The devotees also carry the river water with them and distribute to family member, relatives and also in the community. The river water is also being used to clean the green and leafy vegetables before supply to the market. However, during recent years, this river has been heavily polluted and has become an urban drainage as well as site for waste dumping resulting into river of sewer system.

Environmental surveillance plays an important role in cholera control.[17] Cholera outbreaks can be predicted

by detecting *V. cholerae* O1 and vibriophages in sewage water.[18] In this study, we attempted to isolate the *V. cholerae* O1 and other medically important *Vibrios* in sewerage samples collected from different locations of Kathmandu Valley.

MATERIALS AND METHODS

Sample collection: A total of 42 samples were collected from the different sites during rainy summer season (June to September 2008) (Fig-2) of sewer system of Kathmandu Valley using principle based on Moore's technique. Briefly, this technique involves the cotton gauge (swab) wrapped on one end of a piece of six inch diameter pipe (five inch long) placed horizontally into the sewerage in opposite to sewerage flow for overnight. Samples [Moore's technique based swabs were submersed in alkaline peptone water (broth) pH of 8.6] were transported to Research Laboratory of National Institute of Tropical Medicine and Public Health Research (NITMPHR), Kathmandu in cold condition (ice chest).

Sample processing: The samples were incubated at 37^{0}C for 8 hours followed by culture on thiosulfate-citrate-bile salts-sucrose (TCBS) agar and incubated at 37^{0}C for overnight (15hrs). The TCBS plates showing colonies resembling *V. cholerae* and other *Vibrios* were subjected for identification following standard bacteriological procedures. The organisms were inoculated into triple sugar iron (TSI) agar. The TSI reaction as alkaline or acid slant, acid butt with no gas and H_2S was suspected to be *V. cholerae.* Based on the TSI reaction, suspected colonies were sub-cultured on nutrient agar (NA) and the colonies on NA were subjected for serotyping using polyvalent *V. cholerae* 01 sera and also with Inaba and Ogawa antisera (Denka Seiken Co. Ltd, Japan) as described by Feeley and Balows (1974).[19] For the characterization of other species (*V. cholerae* 01 and other medically important *Vibrios*), colonial characters and biochemical tests (such as sucrose fermentation, lysine utilization, motility, indole test, oxidase positive, swarming, growth on 0, 2, 3, 6% NaCl and others) were employed.

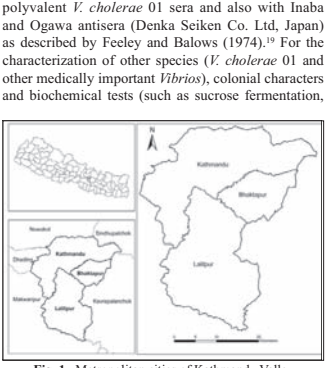

Fig. 1. Metropolitan cities of Kathmandu Valley

Fig. 2. Drainages to Bagmati River (Nadi) showing sampling sites (● indicates sampling sites)

Antibiotic susceptibility test: The *V. cholerae* O1 isolates were subjected to *in-vitro* susceptibility test employing Kirby-Bauer disc diffusion method as recommended by Clinical and Laboratory Standard Institude.[20] Antibiotic discs used in this study consisted of common antibiotics prescribed by physician in Nepal. To differentiate the classical from El Tor cholera species poymixin B disc test was done as described by Han and Khie (1963).[21]

RESULTS

Of the total 42 sewage samples included in this study, 46 medically important *Vibrios* were isolated. Among medically important *Vibrios*, *V. cholerae* was dominant isolates (43.5%) followed by the *V. fulnificus* (23.9%) and other species (Table-1).

Table-1: Frequency of medically important *Vibrios*

***Vibrio* species**	**n**	**%**
V. cholerae	20	43.5
V. vulnificus	11	23.9
V. parahaemolyticus	5	10.9
V. furnissi	5	10.8
V. fluvialis	3	6.5
V. alginolyticus	2	4.3
Total	46	100

Out of 20 *V. cholerae* isolates, 13 (65%) and 7 (35%) isolates were characterized as *V. cholerae* O1 and non

O1, respectively. Among the *V. cholerae* O1, classical Hikojima strain was most frequently isolated followed by the El Tor Ogawa. *V. cholerae* O1 Inaba was not isolated in this study (Table-2).

Table-2: Results of serovar and biogram of *V. cholerae* O1

V. cholerae O1 isolates (n=13)					
Serovar			Biovar		
Type	N	%	Type	N	%
Hikojima	10	76.9	Classical Hikojima	10	76.9
Ogawa	3	23.1	El Tor Ogawa	2	15.4
Inaba	---	----	Classical Ogawa	1	7.7
Total	13	100	Total	13	100

Only one strain (*V. cholerae* O1 classical Ogawa) was multidrug resistant and was resistant to tetracycline, nalidixic acid and chloramphenicol. This strain was isolated from the sample collected from the sewerage outlet draining to *Bagmati River* from the airport and gulf-court side.

DISCUSSION

Three metropolitan cities namely, Kathmandu (capital city), Lalitpur and Bhaktapur are located in Kathmandu Valley. Despite of these metropolises, various waterborne infectious diseases including cholera outbreak occurs time and again resulting into considerable morbidity as well as mortality.[9,15] This is attributed to the drinking of contaminated water and consumption of contaminated foods and vegetables washed in contaminated water of the river system in the valley. The river systems in the valley are heavily polluted with untreated sewerage outlets of the city constituting the major source of contamination. In fact, the sewerage systems in the valley are either very poor and unscientific or non-existent in some areas. Present study, therefore, was done to find out the existence of *V. cholerae* and other pathogenic species of *Vibrios* in the river systems in the valley.

In this study, we employed Moore swab because of its cost effectiveness, effective way to determine the presence of *V. cholerae* in sewage.[17] Many investigators have also used this method for the isolation of medically important *Vibrios* (both cholera and non cholera *Vibrios*) from sewerage/environment and for tracing the source of infection.[17,18] From the 42 sewage samples collected by Moore's technique a total of 46 medically important *Vibrio* species were isolated. Of these, nearly half was *V. cholerae* followed by the *V. fulnificus* and other species. This finding was lower than those reported from elsewhere during peak rainy days. Nearly similar findings (64.2%) have been reported in Argentina and in Bangladesh.[22, 23] In Japan and India, however, *V. cholerae* were positive in all river water and drinking water samples studied.[24, 25]

We attempted to isolate medically important *Vibrios* during peak rainy season because outbreaks of cholera occur each year with beginning of pre-rainy season and continue to post rainy season.[13-15] Many investigators reported the isolation of *V. cholerae* from the diarrheal fecal samples in Nepal.[13,14] Ono *et al* reported *Vibrio* species as second rank organism among the enteric pathogens detected from the fecal samples.[11] Karki *et al* (2008), reported *V. cholerae* at the rate of 27.1% (210 stool samples detected).[26]

To our knowledge, data for the isolation of *Vibrios* other than *V. cholerae* from clinical samples in Nepal are rare. This is virtual ignorance because *Vibrios* such as *V. vulnificus, V. parahaemolyticus, V. furnissi, V. fluvialis* and *V. alginolyticus* are also associated with causation of some kinds of human illness.[3]

In this study, among *V. cholerae,* 65% (13/20) and 35% (7/20) isolates were characterized as *V. cholerae* O1 and non-O1, respectively. Serologically, this study was limited to detect only *V. cholerae* O1. So, our non-O1 isolates might be biochemically related cholera *Vibrios* such as *V. cholerae* O139 and others. According to the Finkestein (1973), *Vibrios* resembling *V. cholerae* but failing to agglutination in cholera anti-sera, had been strongly implicated as causative agents of both sporadically occurring and focal cholera like diarrheal disease.[27]

In this study, among the *V. cholerae* O1, both Hikojima (all classical biotypes) and Ogawa (El Tor biotypes and Classical) serotypes were isolated. This was in agreement with the previous reports from Nepal.[12] Nepal Public Health Laboratory (NPHL) reported *V. cholerae* O1 sero-types, viz. Ogawa, Hikojima from the clinical cases of Nepal and few cases had been associated with Inaba sero-type.[12] Pokhrel and Kubo reported isolation of mixed serotypes, with the dominance of Hikojima from the diarrheal cases in Kathmandu.[13] However, investigators have reported *V. cholerae*, O1 biotype El Tor Ogawa as the major cause of cholera outbreak in Nepal.[14,15,26,28] In this study, however, Inaba serotype was not detected for which we do not have an explanation.

Emergence of drug resistance strain of *V. cholerae* [26,29,30] is global concern which could be addressed by public health strategies. Cholera outbreak with drug resistant strain could create health havoc to the community. This is even true in Nepal where cholera outbreak occur with considerable numbers of MDR *V. cholerae*.[14, 26, 31, 32] In this study, one strain of *V. cholerae* (O1 classical Ogawa) was multidrug resistant (MDR) (resistant to tetracycline,

nalidixic acid and chloramphenicol). Environmental distribution of MDR *V. cholerae* strain may have strong implication on spread of infection.

In conclusion, the presence of medically important *Vibrios* in sewer system of *Bagmati River* should not be ignored. This is the crucial indication for possible outbreak of cholera and other *Vibrio* infections anytime in future and, therefore, demands proper sanitation, supply / consumption of safe drinking water and practice of personal hygiene.

ACKNOLEDGEMENTS

Authors are thankful to Mrs. Chandra Kala Rai, Shi-Gan Health Foundation, Kathmandu, for her encouragement and support during the study.

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