American Journal of Infectious Diseases and Microbiology, 2015, Vol. 3, No. 5, 141-143
Available online at http://pubs.sciepub.com/ajidm/3/5/1

DOI:10.12691/ajidm-3-5-1

Occurrence of a Cholera Outbreak in Central India

Meena Mishra[1], Arvind Kurhade[2], Yagnesh Thakar[3], Geeta Kurhade[4], Angel Justiz Vaillant[5,*], Sudhir Lakhdive[6], Tejaswini H Joshi[7], Sehlule Vuma[2]

[1]Department of Microbiology, Government Medical College and Hospital, Nagpur, India
[2]Pathology and Microbiology Unit, Department of Para, Clinical sciences, University of West Indies, St. Augustine, Trinidad and Tobago
[3]Consultant Microbiologist, Nagpur, India
[4]Physiology Unit, Department of Preclinical Sciences, University of West Indies, St. Augustine, Trinidad and Tobago
[5]Pathology and Microbiology Unit, Department of Para Clinical sciences, UWI, St. Augustine, Trinidad and Tobago
[6]Professor of Surgery, Great Eastern Medical School, Srikakulum, India
[7]Post Graduate Diploma Program, Defense Services Staff College, Madras University, Wellington, India
*Corresponding author: Angel.Vaillant@sta.uwi.edu

Abstract The aim of this research was to carry out a bacteriological study of *Vibrio cholerae* in Central India. Cholera is an important public health problem in India and it continues to be a major concern as it is an important cause of morbidity mortality. A total of 44 strains of *V. cholerae* were isolated from 150 stool samples received from patients with acute diarrhea. All samples were plated onto different bacteriological media. Biotyping was performed as per the standard procedures. Confirmation of the strains was done by seroagglutination using Polyvalent O1, monospecific Ogawa and Inaba antisera. Antibiotic susceptibility testing was performed by Kirby Bauer's disk diffusion method. The majority of the isolates belonged to type 27 (70.45%, i.e. 31 isolates). All Isolates were susceptible to tetracycline, norfloxacin and were relatively susceptible to gentamicin (95%) and chloramphenicol (95%). Continued monitoring, surveillance of all outbreaks and notification to relevant authorities are of utmost importance in the fight against cholera. In addition, the molecular subtyping was essential to improve the tracing of the sources of the outbreak.

Keywords: *Vibrio cholerae, bacteria, bacteriological media, Antibiotic susceptibility, India*

Cite This Article: Meena Mishra, Arvind Kurhade, Yagnesh Thakar, Geeta Kurhade, Angel Justiz Vaillant, Sudhir Lakhdive, Tejaswini H Joshi, and Sehlule Vuma, "Occurrence of a Cholera Outbreak in Central India." *American Journal of Infectious Diseases and Microbiology*, vol. 3, no. 5 (2015): 141-143. doi: 10.12691/ajidm-3-5-1.

1. Introduction

Vibrio cholerae is a motile, gram-negative, curved rod that belongs to the family Vibrionaceae and is considered an important food- and water-borne bacterial pathogen. There are approximately 200 recognized O serogroups, of which serogroups O1 and O139 are associated with cholera pandemics in humans [1]. Cholera is an important public health problem in India and it continues to be a major concern as it is an important cause of morbidity mortality. It is an endemic disease with epidemics occurring at regular intervals. Cholera is transmitted through fecal contamination of water or food and causes an acute, severe, watery diarrhea that can result in hypovolemic shock and death if not treated with fluids [1].

A national and international response should be prompt and its emphasis should be on minimizing mortality by using oral rehydration, preventing infection by promoting treatment of all water supplies for human consumption, adequate sanitation and hygiene, and safe food preparation [2]. Antimicrobial susceptibility testing of selected *V. cholerae* O1 isolates conducted at the National Laboratory of Public Health and at CDC demonstrated susceptibility to tetracycline (susceptibility to this drug predicts doxycycline susceptibility), ciprofloxacin, and kanamycin; and resistance to trimethoprim-sulfamethoxazole, furazolidone, sulfisoxazole, and streptomycin [2].

Bhattacharya and collaborators reported in 2015 the emergence of new variant form of *V. cholerae* O1 El Tor biotype with a novel mutation in ctxB in strains isolated from various outbreaks during 2010-2014 in Belgaum situated in north-west Karnataka, India. All the *V. cholerae* O1 isolates were subjected to PCR to detect the three different allelic subtypes and 14 strains were subjected to ctxB gene sequence and pulsed-field gel electrophoresis (PFGE) analysis. The authors concluded that this was the first report of the Haitian variant of *V. cholerae* O1 Ogawa causing outbreaks and sporadic cases of cholera in South India [3].

Bacteriophage typing is a convenient and highly discriminatory method of identifying epidemic strains of *V. cholerae*. Constant monitoring of the prevalent phage types in an area is important as introduction of a new phage type may herald the onset of an outbreak [4]. The aim of this research was to carry out a bacteriological study of a cholera outbreak that affected Central India in 2010.

2. Material and Methods

A total of 44 strains of *V. cholerae* were isolated from 150 stool samples received from patients with acute

diarrhea who were admitted to the Government Medical College, Nagpur, between 1 April 2010 and 30 September 2010. The bacterial isolation was carried out using standard laboratory techniques [5] at the Department of Microbiology, Government Medical College of Nagpur, India.

2.1. Stool Isolation and Culture of *V. cholerae*

Alkaline peptone water was used for the preliminary enrichment of vibrios from the feces. All the samples were plated onto MacConkey's agar, Blood agar and Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar plates [5]. The suspected colonies were subjected to Gram stain, oxidase test, and motility and string tests. The Gram negative rods that were oxidase positive, actively motile and string test positive were subjected to further biochemical tests [6]. These were indole, triple sugar iron agar, cholera-red reaction, citrate utilization, ornithine decarboxylase, lysine decarboxylase, arginine dihydrolase and sugar fermentation tests using sucrose, mannitol, arabinose and mannose [5]. Biotyping was performed as per the standard procedures [5]. Confirmation of the strains was done by seroagglutination using Polyvalent O1, monospecific Ogawa and Inaba antisera (Central Research Institute, Kasauli) [5].

Antibiotic susceptibility testing was performed by Kirby Bauer's disk diffusion method [6]. The following commercial antibiotic disks (Hi Media Laboratories, Mumbai) were used: tetracycline (30 µg), ampicillin (10 µg), furazolidone (100µg), cotrimoxazole (25 µg), gentamicin (10 µg), norfloxacin (10 µg) and chloramphenicol (30µg) and E. Coli ATCC 25922 reagent was used as control. The plates were read after 16-18 hours incubation at 37 °C. The zone of inhibition for each antibiotic was interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines. After confirmation, the isolates were stocked in alkaline nutrient agar deeps. All the isolates were sent for phage typing at the National Institute of Cholera and Enteric Diseases, Kolkata, where confirmation of the isolates was performed by standard microbiological techniques [6].

3. Results

During the period from 1 April 2010 and 30 September 2010, a total of 150 stool samples from acute diarrhea cases were processed in our laboratory and from 150 stool samples *V. cholerae* was isolated in 44 (29.33%) and all isolates were *V. cholerae* O1. (Table 1)

Out of the number of patients with acute diarrhea due to *V. cholerae*, 25 were from urban areas and 19 were from nearby villages. This outbreak has almost equally affected rural as well as urban population of this region. Males and females were also equally affected in this outbreak.

All the isolates identified belonged to the Basu and Mukherjee phage type T-2. In the present study, the new phage typing scheme against *V. cholerae* O1 El Tor strains showed the following phage types: T-27, T-23, T-26, T-12, and T-13 as shown in Table 1. The majority of the isolates belonged to type-27 (70.45% i.e. 31 isolates). All isolates were susceptible to tetracycline, norfloxacin and were susceptible to gentamicin (95%) and chloramphenicol (95%) as shown in Table 2.

Table 1. Phage type of *V. cholerae* O1 isolates (n=44)

No. of isolates	Biotype	Serotype	Phage type	
			Basu & Mukherjee	New scheme
31	El Tor	Ogawa	T-2	T-27
5	El Tor	Ogawa	T-2	T-23
3	El Tor	Ogawa	T-2	T-26
3	El Tor	Ogawa	T-2	T-12
2	El Tor	Ogawa	T-2	T- 13

Table 2. Antibiotic sensitivity pattern of *V. cholerae* O1 isolates (n=44)

Antibiotic	Sensitive (%)	Resistant (%)
Gentamicin	42 (95.4)	2 (4.6)
Tetracycline	44 (100)	0 (0)
Furazolidone	14 (31.8)	30 (68.2)
Ampicillin	15 (34.1)	29 (65.9)
Streptomycin	12 (27.3)	32 (72.7)
Co-trimoxazole	3 (6.8%)	41 (93.2)
Chloramphenicol	42 (95.4)	2 (4.6)
Norfloxacin	44 (100)	0 (0)

4. Discussion

Vibrio cholerae O1 El Tor Ogawa has been the offending agent since 1964. However, since 1992, a new strain - *V. cholerae* O139 - first isolated in South India, has been responsible for local outbreaks in geographically distinct areas in India [7]. In this study, the 29.33% is the percentage of isolation among patients with diarrhea. All the 44 isolates were *V. cholerae* O1 and belonged to the biotype El Tor and serotype Ogawa. These was similar to the findings of Shah et al (2012) in their study in 2010 in Jamnager, India [8]. In this study, during this recent outbreak there was not a single isolate of O139. This strain seems to have been completely replaced by *V. cholerae* (El Tor) Ogawa. Re-emergence of O139 in 1998 was observed in South India [9]. In addition, sporadic outbreaks from Ludhiana since 2004 have also been reported [10]. Interestingly, in 2000 Pallavi Gard et al, in Calcutta, India, had shown the Inaba strain to have replaced the Ogawa strain. (Pallavi Gard, 2000) [11].

Phage typing for *V. cholerae* is one of the best established tools and markers for epidemiological characterization of isolates. All of them were identified and they belonged to the Basu and Mukherjee phage type T-2, which is similar to the study reported by Oberoi and Aggarwal in 2007 [10]. These findings are in contrast with several studies from Mumbai [4] and Bikaner, where all the strains belonged to phage type T-4 [12]. The most prevalent biotypes in India are T-2 and T-4 [13]. But, the present study reports that the following four phage types: T-27, T-21, T-25 and T-23 were the most prevalent in Central India [Table 1].

The majority of the isolates belonged to type-27 (70.45%, i.e. 31 isolates). The pattern of phage typing nearly coincides with that of a study carried out in 2009 by Srirangaraj and Venkatesha (78.58% i.e.11 isolates) [14]. T-27 was the predominant phage type reported from studies in Bikaner [12] and Mumbai [4]. In the present

study showed differences among the prevalence of isolates; where 5 (11.36%) belonged to T-23, 3 each (6.81%) belonged to T-26 and T-12 and 2 (4.54%) belonged to T-21. The new scheme was more discriminatory and it could identify five circulating phage types when compared with a single phage type identified by the Basu and Mukherjee scheme.

Tetracycline is the drug of choice for the treatment of cholera. In this study all the isolates were susceptible to tetracycline and norfloxacin. The isolates showed relative susceptibility to gentamicin (95%) and chloramphenicol (95%). Similar susceptibility pattern results of these antibiotics against *V. cholerae* have been reported from a study in Tamil Nadu [13]. Sensitivity to ampicillin (34.1%) and co-trimoxazole (6.8%) was also observed and it is depicted in Table-2. Multidrug resistant *V. cholerae* have been reported in different parts of the India [15-16], making imperative that all isolates must be subjected to susceptibility and resistance patterns. It must be monitored as the emergence of resistance among vibrios may significantly influence the strategies in future outbreaks.

It was reported in the literature that *V. cholerae* strains were isolated from 29 states of India. All of the strains belonged to serogroup O1 biotype El Tor. The predominant phage type was T-27. Multidrug-resistant strains showed resistance to norfloxacin and ampicillin [17]. This contrasts with the results obtained in our study that showed 100% of the *V. cholerae* strains were sensitive to norfloxacin and tetracycline. A study carried out in various regions of Kenya between 2007 and 2010 showed that serotype Inaba was dominant (88.2%) compared to Ogawa. In addition the isolates showed varying levels of antibiotic resistance ranging from 100% susceptible to tetracycline, doxycycline, ofloxacin, azithromycin, norfloxacin and ceftriaxone to 100% resistant to furazolidone, trimethoprim-sulfamethoxazole, polymyxin-B and streptomycin [18].

5. Conclusion

Cholera continues to be a public health challenge. Continued monitoring, surveillance of all outbreaks and notification to relevant authorities are of utmost importance in the fight against cholera. In addition, the molecular subtyping was essential to improve the tracing of the sources of the outbreak. The predominant phage type was T-27 and was sensitive to tetracycline and norfloxacin, and resistant to co-trimoxazole.

Acknowledgement

The authors acknowledge the help provided by National Institute of Cholera and Enteric Diseases (NICED), Kolkata for confirming and phage typing the *V. cholerae* isolates.

References

[1] Riedl J, Klose KE: 2002, Vibrio cholerae and cholera: out of the water and into the host. FEMS Microbiol Rev 26:125-139.

[2] Centers for Disease Control and Prevention (CDC). Cholera outbreak --- Haiti, October 2010. MMWR Morb Mortal Wkly Rep. 2010; 59(43):1411.

[3] Bhattacharya D, Dey S, Pazhani GP, Ramamurthy T, Parande MV, Kholkute SD, Roy S. Vibrio cholerae O1 El Tor variant and emergence of Haitian ctxB variant in the strains isolated from South India. Med Microbiol Immunol. 2015 Sep 4. [Epub ahead of print].

[4] Turbadkar SD, Ghadge DP, Patil S, Chowdhary AS, Bharadwaj R. Circulating phage type of *Vibrio cholerae* in Mumbai. Indian J Med Microbiol 2007; 25:177-8.

[5] Collee JG, Fraser AG, Marmion BP, Simmons A. Mackie and McCartney- Practical Medical Microbiology, 14 th ed. Churchill Livingstone: New Delhi; 2006. p. 425-8.

[6] Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology, WHO, Geneva, 2 nd ed. Delhi: AITBS Publishers; 2004. p. 41-54.

[7] Albert MJ. Epidemiology and molecular biology of *Vibrio cholerae* O139 Bengal. Indian J Med Res 1996; 104:14-27.

[8] Shah HD, Shah VP, Desai AN An epidemic outbreak of Vibrio Cholerae El Tor 01 serotype ogawa biotype in a Lalpur town, Jamnagar, India. Journal of post grad med 2012 Jan-Mar; 58(1):14-8.

[9] Raut S, Jalgaonkar N, Tankhiwale S , Agrawal V. Re-emergence of Vibrio cholera O139 during 1998 in Nagpur , India. Indian J Med Res 1999; 109: 1-2.

[10] Oberoi A, Aggarwal A. Changing serotypes and phage types of *Vibrio cholerae* in Ludhiana (Punjab) during 2001-2005. Indian J Med Microbiol 2007; 25:75-6.

[11] Garg P, Nandy RK, Chaudhury P, Chowdhury NR, De K, Ramamurthy T, Yamasaki S, Bhattacharya SK, Takeda Y, Nair GB. Emergence of Vibrio cholerae O1 biotype El Tor serotype Inaba from the prevailing O1 Ogawa serotype strains in India. J Clin Microbiol. 2000; 38: 4249-53.

[12] Gupta A, Jain S, Mahawal BS. Outbreak of cholera in arid zone of Bikaner. Indian J Med Res 1999; 110:126-7.

[13] Sundaram SP, Revathi J, Sarkar BL, Bhattacharya SK. Bacteriological profile of cholera in Tamil Nadu (1980-2001). Indian J Med Res 2002; 116:258-63.

[14] Srirangaraj S, Venkatesha D. Circulating phage type of *Vibrio cholerae* in Mysore. Indian J Med Microbiol 2009; 27:166-7.

[15] Taneja N, Mohan B, Khurana S, Sharma M. Antimicrobial resistance in selected bacterial enteropathogens in North India. Indian J Med Res 2004; 120: 39-43.

[16] Mathur M, De A, Saraswathi K, Varaiya A, Athalye S. Vibrionaceae from cases of acute diarrhea and their antimicrobial sensitivity pattern- A five year prospective study. Indian J Med Microbiol 2003; 21: 199-201.

[17] Jaiswal A, Sarkar S, Das P, Nandy S, Koley H, Sarkar B. Trends in the genomic epidemiology of Vibrio cholerae O1 isolated worldwide since 1961. Int J Antimicrob Agents. 2015 Jul 22. pii: S0924-8579(15)00250-2.

[18] Mercy N, Mohamed AA, Zipporah N, Chowdhury G, Pazhani GP, Ramamurthy T, Boga HI, Kariuki SM, Joseph O. Phenotypic and genetic characterization of Vibrio cholerae O1 isolated from various regions of Kenya between 2007 and 2010. Pan Afr Med J. 2014; 19:8.