Research Article

Trend of Antimicrobial Susceptibility Profile of Vibrio cholera Strains Isolated in Indian Children's during 2008-2016

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A B S T R A C T

Background: The unique epidemiologic attribute of the cholera is its propensity to occur as an outbreak that may flare- up into epidemics, if not controlled. The causative bacterial pathogen Vibrio cholerae prevails in the environment and infects humans whenever there is a breakdown in the public health component. The upsurge in antimicrobial resistance directly influences the management and spread of the disease.

Objectives: The present study documents the epidemiological profile and the changing trends in antimicrobial resistance of eight years (2008-2016) in Vibrio cholerae isolates.

Methodology: A retrospective study was undertaken with review of records of a period of eight years (January 2008 to December 2016) from two government hospitals in Delhi. All data were captured in WHONET and was analyzed. V. cholerae isolates were identified using standard microbiological techniques and were serotype using antisera. Antimicrobial susceptibility testing was performed using disc diffusion and Vitek-2 automated method.

Result: During the period of eight years, 315 cases were confirmed microbiologically as cholera. A significant outbreak of cholera (88 cases) occurred in 2013 followed by sporadic cases in 2012 through 2016. Males outnumbered the females by Male to female ratio of 1:0.8. Mostly cases presented during the months of June to October. Almost all (92%) isolates were V. cholerae O1, biotype ElToR and serotype Ogawa. The antibiogram over the period of eight years showed that isolates were consistently sensitive to aminoglycosides. However, emerging resistance was seen to quinolones and β-Lactam group.

Conclusion: The emergence of resistance amongst V. cholerae especially towards quinolones may significantly influence the control strategies in future outbreaks. Therefore, continuous surveillance with regards to drug resistance, as well as epidemiological variation is necessary for early detection. A strong regional commitment may help contain the disease.

Keywords: Antimicrobial Resistance, Vibrio Cholerae, Cholera Outbreak, Delhi

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Introduction

Cholera is a global health problem as several thousand of cases and deaths occur each year.[1] Global burden of cholera is huge, particularly in developing countries. Every year an estimated 2.8 million cases of cholera (uncertainty range: 1.2-4.3 million) and about 91,000 deaths (uncertainty range: 28,000-142,000) occur in endemic countries and another 87,000 cases and 2500 deaths occur in non-endemic countries.[2] The burden of cholera is greatest in Africa and Southern Asia.[3]The incidence of cholera is estimated to be 1.6 cases/1000 population per year or 40 per 1000 cases of acute diarrhea in India.[3] India has 1% of the world's cholera burden with average 170,000 cases and over 1300 deaths due to cholera reported annually.[4]

The causative organism is essentially a food and waterborne Gram-negative comma-shaped bacillus, the peak transmission rates of which coincide with monsoon season and outbreaks are frequently noted in areas of inadequate sanitation services and poor access to clean water supply.[3,4]

Outbreak of cholera in Yemen has reported a cumulative total of 17,334 suspected cases of cholera, including 99 associated deaths across the country, with a case-fatality rate of 0.6%.[5] Of these, 189 cases were laboratory-confirmed for Vibrio cholerae (V. cholerae) 01 in Yemen.[5] Around the same time, in Somalia the cholera outbreak has seen a cumulative total of 51,036 cases including 782 deaths.[6] Constant change in the biotype and serotypes of V. cholerae is an also important aspect that alters virulence and survival of the pathogen.[7]

The antimicrobials commonly used in the treatment of cholera include tetracycline and fluoroquinolones, such as ciprofloxacin and nalidixic acid.[8] The antimicrobial susceptibility patterns of V. cholerae and antibiotic resistant strains from newly-infected patients are on constant change following the recent emergence and spread of Multidrug-Resistant (MDR) strains.[9] In view of the reported shift in epidemiology and pattern of antibiotic resistance in this was study carried out to assess the development of resistance to essential drug like fluoroquinolones during treatment of cholera and cholera like cases in Delhi. Keeping this in view and the limited availability of contemporary data in this regard over the last few years, the present study was undertaken as an attempt to document the epidemiological and antimicrobial susceptibility profile of V. cholerae strains isolated at two Indian tertiary care hospitals in Delhi over a period of eight years.

Materials and Methods

A retrospective, observational, laboratory based study from January 2008 to December 2016, was conducted in two hospitals of Delhi, India. One hospital is situated in Eastern part of Delhi (Chacha Nehru Bal Chikitsalaya; 216 bedded pediatric hospital) and another is in the central part of the Delhi (Maulana Azad Medical College and associated 2000 bedded-Lok Nayak hospital). During the study period a total of 10,777 fecal specimens of children aged between 2 months to 12 years of age were included in the present study. All data was captured in WHONET software and was analyzed.

The stool specimens were cultured as per standard protocol.[10] Stool samples were observed for macroscopic findings and a hanging drop preparation was made and examined for darting motility. In addition, a stool wet mount examination for red blood cells, pus cells, cysts, and trophozoites of parasites was performed. The stool samples were streaked onto MacConkey agar, Thiosulfate-Citrate-Bile Salts-Sucrose agar (TCBS) and deoxycholate citrate agar (DCA), and incubated at 37°C for 18-24 h. A part of the stool samples was enriched in alkaline peptone water and selenite F broth at 37°C for 6 h, and subsequently subcultured onto TCBS and DCA, respectively. Any typical V. cholera like colonies were further characterized by conventional biochemical tests. The isolated strains were identified using Vitek 2 compact system Biomeriux, India. Isolates with biochemical reactions resembling those of Vibrio were confirmed serologically by slide agglutination test employing specific antisera (polyvalent O1, O139, and monospecific Ogawa and Inaba antisera) obtained from Denka Seiken Company Limited, Tokyo, Japan.

All isolated strains of V. Cholerae were tested for in vitro antimicrobial susceptibility test as per Clinical and Laboratory Standards Institute (CLSI) guidelines.[11] Quality control was achieved using standard strain of Escherichia coli ATCC 25922. A panel of antimicrobial agents was used for susceptibility testing. These included ampicillin (10 µg), ciprofloxacin (5 µg), tetracycline (30 µg), amikacin (30 µg), ceftriaxone (30 µg) and ampicillin (10 µg).

Monthly data for rainfall and temperature was collected from the website of Indian Meteorological Department (Ministry of Earth Sciences), Government of India for the period of 2008 through 2016.[6]

Statistical Analysis

Data analysis was performed using statistical software, version 3.5.3, Centers for Disease Control and Prevention, Atlanta, GA, USA. Descriptive statistics were expressed in terms of proportions and figures. Any differences in resistance patterns across the years were determined using Fisher's exact test and Chi-square test, and a two-tailed P <0.05 was considered as statistically significant different.

Result

A total of 315 (1.36%) laboratory confirmed cholera patients were diagnosed. The present study revealed incidence density of 12.6 cases per 1000 diarrheal cases in this region.

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Incidence density of V. cholerae from central Delhi hospital was 1% (n=9577) while, East Delhi hospital were 0.36 % (n=1200). In present study males outnumbered the females by M: F=1:0.8. (Table 1) Older children (>5 years) were found to be affected more than younger age group. Statistical significant association was noted between the age and gender groups. (p= 0.02). Most isolates (92%) of V. cholerae O1, biotype ElToR, serotype Ogawa.

Table 1.Age and sex profile of Vibrio Cholerae patients from 2008 to 2016

Age	Male (169)	Female (146)	Total (315)
Below 1 year	30	23	53
>1 years to 5 years	58	37	95
>5 years	81	86	167

Throughout the study period many sporadic cases (range from 25 to 35 cases/ year) were seen during specific months of the year. Occurrence of cases followed a typical seasonal pattern. All the cases were clustered in the rainy season (June through September) every year. During this time period the rainfall ranged from 25 to 85.3 mm and temperature from 18°C upto 28°C. Additionally, there was a significant surge of cholera cases was observed in the year 2010 (110 cases/ year) and 2013 (88 cases/ year). In these years there was positive correlation emerged with higher rainfall (rainfall range 237 to 278 /mm) and higher temperatures (32°C upto 42°C) during the rainy season (Figure 1).

Antimicrobial resistance profile of V. cholerae strains across these years is summarized in Table 2. The present study antibiogram reveals that the isolates were consistently susceptible to cephems, penicillin, azithromycin and chloramphenicol. However, reduced susceptibilities were observed to quinolones, tetracycline, aminoglycosides and β-lactam group of antibiotics (Table 2).
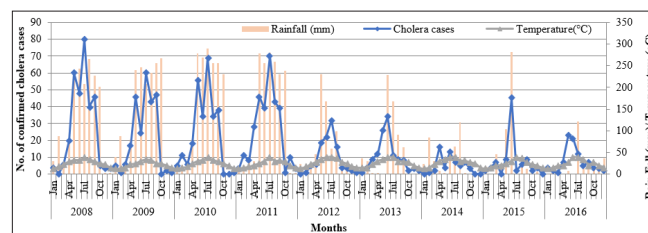

Figure 1.Month-wise distribution of rainfall and temperature with seasonal trends of V. cholera isolates between January 2008 to December 2016

A temporal comparison of antimicrobial resistance profile of V. cholerae strains against some of the commonly used antibiotics, over a period of eight years is summarized in figure 2. Analysis of this antibiogram shows following proportion of isolates resistant to tetracycline (64%-80%), ceftriaxone (10%-40%) amikacin (10%-40%), ampicillin (15%-30%), ciprofloxacin (20%-40%) and erythromycin (12%-36%).

Table 2.Antimicrobial susceptibility profile of V. cholerae (n=315) isolates during 2008-2016

Antibiogram	N	Susceptible (n)	Percent Susceptible (95% C.I.)	MIC50 (μg/ml)	MIC90 (μg/ml)	MIC Range (μg/ml)
Ampicillin (10 μg)	315	304	96.5 (66.3-90.4)	16	32	2-32
Amoxicillin (10 μg)	158	122	77.0 (68.0-79.3)	8	32	2-32
Piperacillin/ Tazobactam (100/ 10 μg)	315	294	93.5 (1.7-18.9)	1	1	1-1
Ceftriaxone (30 μg)	301	285	94.0 (3.9-29.1)	1	1	1-64
Cefotaxime(30 μg)	315	260	82.0 (12.0-21.5)	0.25	0.5	0.047-2
Amikacin (30 μg)	200	160	80.0 (4.2-11.7)	2	4	2-16
Gentamicin (30 μg)	150	98	65.0 (5.3-12.6)	1	4	1-16
Tetracycline (30 μg)	220	156	71.0 (0.4-37.9)	1	1.25	1-20
Ciprofloxacin (5 μg)	315	295	93.6 (12.9-22.8)	0.5	1	0.25-22
Chloramphenicol (30 μg)	315	310	98.4 (2.5-43.9)	8	16	0.25-256
Erythromycin (30 μg)	230	198	86.0 (0.0-60.4)	16	16	1-16
Nalidixic acid (30 μg)	315	312	99.0 (76.5-97.8)	32	32	4-32
Trimethoprim/ Sulfamethoxazole (1.25/ 23.75 μg)	285	215	75.4 (0.0-94.5)	384	384	6-400

*N= No of strain subjected to the drug susceptibility. n= No of susceptible strain for the antimicrobial.

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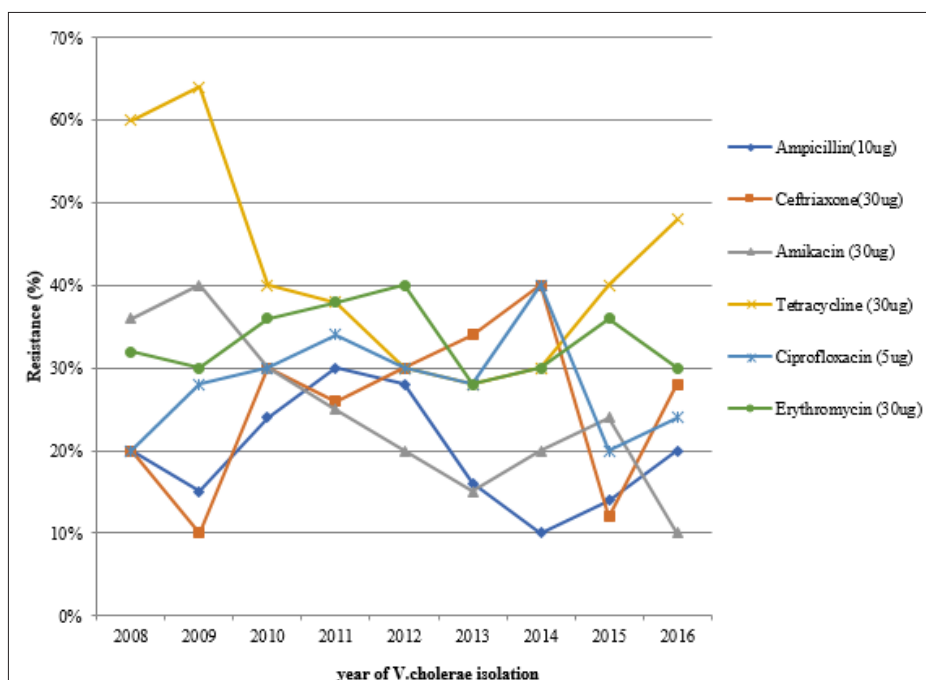*J. Commun. Dis. 2021; 53(1)*

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Figure 2. Year wise comparison of drug resistance pattern of V. cholerae isolates (2008-2016)

Discussion

Cholera has shown a constant endemicity in Delhi region for last several years.[6,7] In the present study, overall prevalence of V. cholerae was 1.36% cases which were lower as compared to the previous studies, which have reported prevalence of 5.79% to 9.6% in other parts of India.[8-10] High incidence in endemic areas has been reported by other studies and drastically higher detection rates are documented in outbreak situations. As reported in other studies conducted in different parts of India, current study also had most of cases (53%) being reported in children more than five years in age.[11-13] The seasonal pattern observed is similar to the one reported from Eastern, Western and Northern part of India.[7-9] The occurrence of cases correlated well with the onset of monsoon in this region. Mostly all the isolates were V. cholerae O1, biotype El TOR serotype. The present finding corroborated well with study by Sarkar et al.[14] This suggests that a particular clone of V. cholerae O1 strain is probably circulating all over India. Therefore, over the period of eight years antibiogram showed that isolates were consistently sensitive to gentamicin, amikacin, ciprofloxacin and cefotaxime. However, resistance was seen with amikacin (9.1%) and ceftriaxone (4%). These findings coincides with other studies which also reported isolates being sensitive to gentamicin and tetracycline.[14,15] The resistance profile of V. cholerae is known to show variations, depending on the local antibiotic over use/abuse at that period of time. Use of antimicrobial agents is generally accepted as a method of reducing the duration and volume of diarrhea as well as decreasing the period of V. cholerae excretion in stool.[16-18] Hence, even resistance

of a third-generation cephalosporin, ceftriaxone (4%) is significant and requires monitoring. The antibiotic resistance pattern of epidemic strains has changed frequently with the emergence of different V. cholerae O1 or O139 strains. Therefore selection of such drug resistant clones can lead to seasonal epidemics of cholera with emergence of new clones replacing the existing clones. Synchronized monitoring of such clinical as well as environmental strains thus becomes essential in order to understand the cloned spread of these novel multi drug resistant strains in the cholera endemic areas.

Thus, we observed that a significant proportion of V. cholerae strains at our center are no longer sensitive to the commonly used antibiotics for the treatment of enteric infections. Furthermore, the current data have shown that fluctuations in antibiograms of clinical isolate of V. cholerae are common, and on comparing our findings with the published reports from Delhi and other parts of the country, we found that wide temporal and spatial variations are noted in the antibiotic treatment protocols and thus resistance patterns often differ by geographical region. This implies that antibiotic sensitivity pattern of V. cholerae cannot be predicted easily and it is imperative that each and every clinical isolate should be subjected to antimicrobial susceptibility testing. Furthermore, resistance patterns fluctuate with variations in the serogroups and this thus mandates not only a continuous vigilance of the changing resistance profiles of the isolates but also monitoring of cyclical changes in the circulating serovars and serotypes in the population.

Our analysis has provided recent insight into the

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epidemiology and resistance profile of V. cholerae strains isolated at one of the largest tertiary care institutes in North India. The study, however, is not without limitations. In addition, genetic analysis of isolates to determine the genomic basis of resistance to various classes of antimicrobials and molecular studies to determine their clonal type was not performed. Our study has provided a more contemporary epidemiological profile of cholera in this region and has emphasized on the rapid gain and increase in the spectrum of antimicrobial resistance among clinical isolates of V. cholerae in this belt that has complicated the selection of antimicrobial therapy and rendered the treatment of cholera enormously difficult. Emerging AMR is a challenge. The study was retrospective in nature and thus clinical information of the cholera affected patients could not be retrieved.

Conclusion

Continuous surveillance of the changing trends in antimicrobial resistance is essential for V. cholerae and the microbe which has potential of causing severe outbreak. Cholera is endemic in this part of world and based on the seasonal trends, limited surge of cases is being observed in most of the years. Strong regional commitment to surveillance and preparedness for outbreaks should be maintained and timely information should be given to the health authorities as well as to the public to contain the spread of this dreaded disease. V. cholerae have a permanent existence in the environment and during the quiescent period, their survival in water bodies allows dissipation of resistance patterns to different serotypes or strains of V. cholerae O1 and therefore there is need for constant observation.

Conflict of Interest: None

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