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Acute Diarrhoea Due to *Vibrio cholerae* and Antimicrobial Susceptibility Pattern in a Tertiary Care Hospital

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Abstract: Acute diarrhoeal disease caused by Vibrio cholerae in many developing nations poses a great public health problem which can kill within hours if left untreated. The present study was undertaken to determine the prevalence of cholera and antimicrobial susceptibility pattern of Vibrio cholerae in a tertiary care hospital at Hyderabad between 2013 and 2017. Design: Retrospective cross-sectional study. Methods: Data of stool samples between 2013 and 2017 from cases of acute diarrhea was analyzed. Stool samples were inoculated onto routine & selective media before and after enrichment. Suspected colonies were identified by standard microbiological techniques and biotyping & serotyping was done. Antimicrobial susceptibility testing was done by Kirby Bauer disk diffusion technique. Stool culture data and susceptibility pattern were analyzed and presented. Results: A total of 133 isolates of Vibrio cholerae were obtained from 6441 stool samples (2.06%), of which 34 (2.73%) were obtained in 2013, 33(2.68%) in 2014, 17(1.13%) in 2015, 47(3.02%) in 2016 and 2(0.21%) in 2017. Maximum isolation was seen between April and August with a peak incidence in July. Out of 133 isolates, 112 (84.2%) isolates were from adults and there was female (52.6%) preponderance. All isolates were Vibrio cholerae O1 El Tor of which Ogawa comprised 116(87.2%), Inaba 17(12.8%) and Hikojima nil. Isolates showed sensitivity to Ceftriaxone (99.2%), Amikacin (99.1%), Cefotaxime (98.4%), Tetracycline (97.0%) and least sensitivity to Cotrimoxazole (3.0%) & Furazolidone (2.4%). Conclusion**:** The cholera incidence was reduced from 2013 to 2017 with a higher isolation in 2016 compared to other years and maximum were isolated in early monsoon. Vibrio cholerae O1 El Tor Ogawa was most common serotype. Most of the isolates were resistant to cotrimoxazole and furazolidone. This emphasizes continued microbiological surveillance and epidemiological studies due to changing trends in antimicrobial susceptibility pattern.
Keywords: Cholera, antibiotic susceptibility, prevalence, Vibrio cholerae, acute diarrhoea.

INTRODUCTION

Vibrio cholerae, the causative agent of the acute diarrhoeal disease cholera, is still a great public health problem. Each year 1.3 million to 4.0 million cases of cholera and 21,000 to 1,43,000 deaths are reported worldwide due to cholera [1]. In Asia, Indian subcontinent contributes about 78 per cent of cholera cases [2]. WHO estimates that the officially reported cases represent only 5–10% of the actual number occurring annually worldwide [3].

Diarrhea is an alteration in a normal bowel movement characterized by an increase in water content, volume, or frequency of stools. A decrease in consistency and an increase in frequency of bowel movements to 3 stools per day have often been used as a definition for epidemiological investigations. Acute diarrhea is an episode of diarrhea lasting less than 14 days [4]. The time taken by a patient to show symptoms after ingesting contaminated food or water is between 12 hours and 5 days [5]. Cholera affects both children and adults and can kill within hours if not treated. Severely dehydrated patients are at risk of shock and death. They require rapid administration of intravenous fluids. These patients are also given appropriate antibiotics to decrease the duration of diarrhoea, reduce the volume of rehydration fluids needed, and shorten the amount and duration of *V. cholerae* excretion in their stool. Mass administration of antibiotics is not recommended, as it has no proven effect on the spread of cholera and contributes to increasing antimicrobial resistance [1]. Safe water and sanitation is important to

control the transmission of cholera. Oral cholera vaccines are also used in addition to safe water and sanitation to control and prevent cholera outbreaks [1]. Industrialized countries have seen practically no cholera cases for over a century because of their good water and sewage treatment infrastructure. However, the causative agents (Vibrio cholerae O1 and O139) continue to thrive wherever crowded housing conditions exist and water and sanitation facilities are suboptimal [3]. WHO launched a global strategy in 2017 on cholera control - "Ending Cholera: A Global Roadmap to 2030", with a target to reduce cholera deaths by 90% by 2030.

MATERIALS AND METHODS

At the tertiary care hospital, data of stool samples obtained from cases of acute diarrhea between 2013 and 2017 was analyzed. From 6441 cases of acute diarrhoea stool samples were collected in a sterile container. A loopful of sample was inoculated onto MacConkey agar & TCBS agar. Remaining sample was inoculated in alkaline peptone water and incubated at 37^{o} c for 6 – 8 hrs. After enrichment it was sub cultured onto nutrient agar(NA), bile salt agar(BSA) and TCBS agar and incubated at 37 ^{o} c for 24-48 hrs. After incubation period (24hr for BSA&48hr for TCBS), the plates were observed for the presence of translucent (on BSA) or yellow button shaped colonies (on TCBS). The colony was identified as V. cholerae if it was sucrose fermenting yellow colony on TCBS, glistening translucent on BSA, late lactose fermenting on MA, oxidase positive, catalase positive, urease negative, glucose and sucrose fermentative, maltose non-fermentative, citrate positive, indole positive, methyl red negative, Voges–Proskauer positive/ negative, hydrogen sulphide (H2S) negative, and in triple sugar iron agar tube acid/acid with no gas production & string test positive (identification of V. cholerae CDC, 2012). Biotyping was done by polymyxin B (50 U) sensitivity and chick RBC agglutination. Serotyping was done by slide agglutination with specific antisera (obtained from KIPM, Guindy, Chennai) performed on a glass slide by emulsifying the growth in a small drop of physiological saline and mixed thoroughly. Small drop of antiserum was added to the respective suspension. The suspension and antiserum were mixed well and tilted back and forth to observe for visible agglutination. One drop of physiological saline and the growth emulsion was used as a negative control to observe for auto-agglutination. Antibiotic sensitivity was done by Kirby Bauer disk diffusion method according to CLSI guidelines. A standard dilution of the test isolate was prepared by matching it with 0.5 McFarland turbidity standards and was uniformly swabbed over the Mueller Hinton agar (MHA) medium. Standard strain of Escherichia coli ATCC 25922 was used as control strain. Then antibiotic disks were placed on the medium and incubated at 37 °C for 18-24 h. After incubation period the zones of inhibition were measured and results were interpreted as per the guidelines given by the Clinical& Laboratory Standard Institute (CLSI) [10]. Antibiotic disks used were Amikacin (30 mcg), Gentamicin (10mcg), Tetracycline (30 mcg), Furazolidone (50mcg), Doxycycline (30 mcg), Azithromycin (15mcg), Cefotaxime (30mcg), Ceftriaxone (30mcg), Ciprofloxacin (5mcg), Norfloxacin (10mcg), Ofloxacin (5mcg) & Cotrimoxazole (1.25/23.75 mcg) on two MH agar plates. The culture media and antibiotic disks used were obtained from Hi-Media Ltd, Mumbai.

Year-wise proportions of cholera cases by age, gender, season and antibiotic resistance profile were analysed to determine the trend.

RESULTS

Fig-1: Yearly distribution of cases

Out of the 6441 stool samples obtained from acute diarrheal patients 133 isolates of *Vibrio cholerae* were reported from January 2013 to December 2017(2.06%), of which 34 (2.73%) were reported in 2013, 33 (2.68%) in 2014, 17 (1.13%) in 2015, 47 (3.02%) in 2016 and 2 (0.21%) in 2017(Figure 1). Maximum isolation was seen between April and August with a peak in July (Figure 2). 58 (43.6%) isolates were from 20 – 39yr age group, 34 (25.6%) were from 40 – 59yr age group, 31 (23.3%) were from 1 – 19yr age group and 10 (7.5%) were from age group of 60yr or above (Table1). Out of 133 isolates, 112 (84.2%) isolates were from adults and there was female (52.6%) preponderance (Figure 3). All isolates were Vibrio

cholerae O1 El Tor of which Ogawa comprised 116(87.2%), Inaba 17(12.8%) & Hikojima nil. Isolates showed highest sensitivity to Ceftriaxone (99.2%) followed by Amikacin (99.1%), Azithromycin (99%), Cefotaxime (98.4%), Gentamicin (97.9%), Doxycycline (97.5%), Tetracycline (97.0%) Ciprofloxacin (96.6%), Norfloxacin (96.3%), Ofloxacin (91.8%) and least sensitivity to Cotrimoxazole (3.0 %) & Furazolidone (2.4%) (Table2).

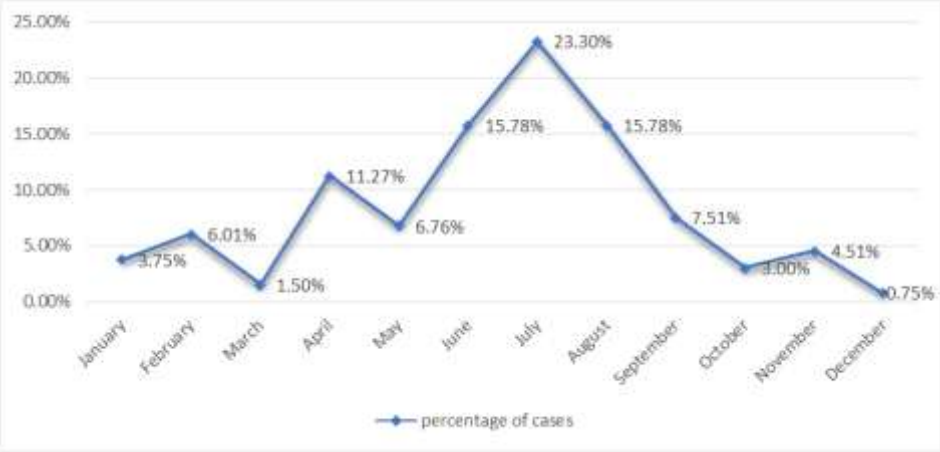

Fig-2: Monthly distribution of cases (2013 -2017)

Table-1: Age wise distribution of cases

Age group	Percentage of isolates
1 – 19 years	23.3%
20 – 39 years	43.6%
40 – 59 years	25.6%
60 years & above	7.5%

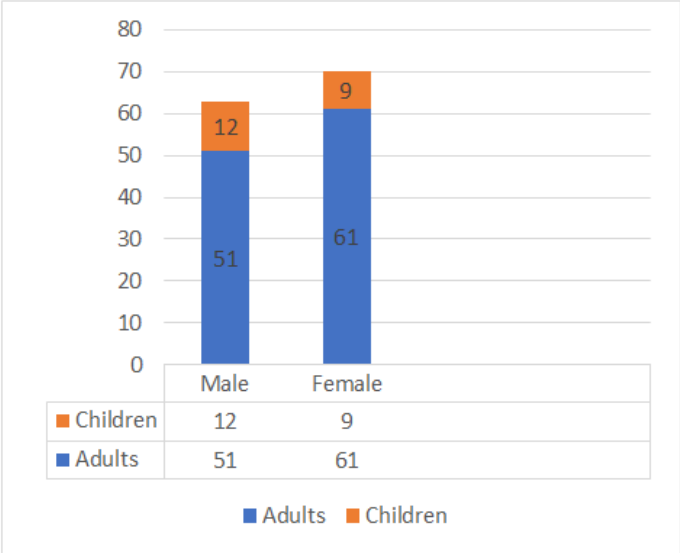

Fig-3: Demographic data

Table-2: Susceptibility pattern of antibiotics

Drug &Year	Ceftriaxone	Amikacin	Azithromycin	Cefotaxime	Gentamicin	Tetracycline	Doxycycline
2013	100%	100%	100%	100%	100%	100%	100%
2014	96.8%	100%	100%	100%	100%	100%	100%
2015	100%	92.8%	100%	93.3%	100%	92.8%	87.5%
2016	100%	100%	97.6%	100%	97.5%	94.6%	95.7%
2017	100%	100%	100%	50%	0%	100%	100%
Total	99.2%	99.1%	99%	98.4%	97.9%	97.0%	96.6%

Drug &Year	Ciprofloxacin	Norfloxacin	Ofloxacin	Cotrimoxazole	Furazolidone
2013	100%	100%	100%	2.9%	0%
2014	96.4%	100%	100%	0%	0%
2015	92.3%	100%	100%	0%	0%
2016	95.5%	90.5%	70%	6.4%	7.5%
2017	100%	100%	100%	0%	0%
Total	96.6%	96.3%	91.8%	3.0%	2.4%

DISCUSSION

Present study was conducted in a tertiary care teaching hospital in Hyderabad. The average prevalence of cholera at this referral centre for infectious diseases was found to be 2.06% in the last five years. Sharma et al observed a prevalence of 3.93% in Guwahati during their period of study [2]. Similar findings were observed by Palewar *et al*. in Pune (4.4%) & Maharjan et al in Nepal (3.28%) [6,7]. But a higher prevalence was observed in Mumbai (9.14% by Torone V *et al*.) [8].

Out of 133 isolates of *Vibrio cholerae* 34 (2.73%) were reported in 2013, 33 (2.68%) in 2014, 17 (1.13%) in 2015, 47 (3.02%) in 2016 and 2 (0.21%) in 2017.The prevalence of cholera appears to decrease from 2013 to 2017 but for the peak in 2016.The study of the epidemiology of cholera outbreaks showed that there was no readily discernible pattern [9] (Figure 1).

Maximum isolation of Vibrio was seen in early monsoon between April and August with a peak in July. Maximum of cases were detected during early monsoon in other studies also [6,8,9]. The level of surface water increases with rains and there will be mixing stagnant water with drinking water through broken pipelines. Such water sources are used by slum dwellers and people in suburban area for cooking and drinking which enhances the chances of infection [9]. The cases were detected throughout the year but peaked in the month of July for all the years and tapered from September onwards. (Figure2).

112 (84.2%) isolates were from adults and 21 were from children (15.8%). The proportion of adults is similar to other workers (71.3%, 72%) [6,8]. Patients in age group 20-39yrs (43.6%) were more affected followed by 40-59yrs (25.6%), 1-19yrs (23.3%) & 60yrs above (7.5%). The cause of high number of patients in age group 20–39 yr may be due to the fact that they belong to working age group and the necessity of taking unhygienic food and water often may have caused the enteric disease [7].

Female (52.6%) preponderance (Figure 3) was observed. Sharma et al also observed an overall female preponderance during their study period. In few other studies male preponderance was seen [9, 7] There is sufficient evidence to say that there was no association between sex and the incidence of the disease ($P > 0.05$).

All isolates were Vibrio cholerae O1 El Tor. The El Tor biotype of V. cholerae has completely replaced the classical biotype. The results of this study confirm these reports. Ogawa comprised 116(87.2%), Inaba 17(12.8%) & Hikojima nil. Predominant serotype was Ogawa (93.26%) with an occasional isolation of Inaba (6.74%) in a study by Torone *et al*. [8]. Sharma *et al*. observed 94.3% of Ogawa isolates &2.9% of Inaba. Palewar *et al*. found 98% of the isolates to be Ogawa.

Isolates showed highest sensitivity to Ceftriaxone (99.2%) followed by Amikacin (99.1%), Azithromycin (99%), Cefotaxime (98.4%), Gentamicin (97.9%), Doxycycline (97.5%), Tetracycline (97.0%) Ciprofloxacin (96.6%), Norfloxacin (96.3%), Ofloxacin (91.8%) and least sensitivity to Cotrimoxazole (3.0 %) & Furazolidone (2. 4%).Year wise sensitivity pattern is given in Table 2. Sensitivity pattern of antibiotics had shown fluctuating trends between 2013 and 2017.In 2013 all the isolates were 100% sensitive to all the antibiotics tested except Cotrimoxazole (2.9%) & Furazolidone (0%). Vibrios have become 100% resistant to cotrimoxazole and furazolidone by 2015. Isolates became sensitive to these 2 drugs to some extent in 2016(6.4% and 7.5% respectively). In 2017 there were only 2 isolates which were 100% sensitive to all the drugs tested except Cefotaxime (50%), Gentamicin (0%), Cotrimoxazole (0%) and Furazolidone (0%).

Antimicrobial resistance (AMR) is one of the greatest challenges to public health locally and globally and has gained particular concern in the developing world. Underuse, misuse and overuse of antibiotics without health care professional's advice, availability of antibiotics even in local groceries, use of antibiotics in farm and field, sharing of antibiotics with family and friends are common, all of which accelerate the development of antimicrobial resistance [7]. Antibiotics are considered as useful additives for the treatment of cholera as these shorten the duration of hospital stay, stop excretion of bacteria in the stool and also minimize the requirement for IV fluids. Even a low level of resistance is significant which requires monitoring because the resistance profile of V. cholerae show variations, based on the local antibiotic over use or abuse at that period of time [9]. Development of resistance to commonly used antibiotics indicates a serious public health concern because it complicates

treatment by extending the duration of hospital stay for patients.

We need adequate prevention and control measures to overcome the impact of cholera. Inadequate reporting of cases is a problem because it can lead to insufficient allocation of resources to effectively deal with cholera. The data presented here may help policy-makers and the WHO's Global Task Force on Cholera Control to determine how much investment future cholera control interventions will require. This also highlights the need to improve cholera surveillance, especially among at-risk populations in endemic countries [3].

CONCLUSION

The prevalence of cholera has decreased from 2013 to 2017 with a higher incidence in 2016 compared to other years and maximum isolates were seen in early monsoon. Vibrio cholerae O1 El Tor ogawa was most common serotype. Antimicrobial Susceptibility pattern of V. cholerae has been changing. Continued microbiological surveillance and epidemiological studies are essential because of these changing trends to control outbreaks of cholera in this region.

This study of cholera burden may aid in developing strategies for reducing endemic and epidemic cholera in the face of climatic, environmental changes and drug resistance and may progress towards improving water supply and sanitation in endemic countries.

APPENDIX

ABBREVATIONS

- AMR – Antimicrobial resistance
- AST – Antimicrobial Susceptibility Testing
- APW – Alkaline Peptone Water
- BSA – Bile Salt Agar
- CDC – Centre for Disease Control
- CLSI – Clinical and Laboratory Standards Institute
- MA – MacConkey Agar
- MHA – Mueller Hinton agar
- NA – Nutrient Agar
- TCBS – Thiosulphate Citrate Bile Salt Sucrose agar

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