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The frequency of vibrio cholera 01 EL TOR (Inaba And Ogawa) and its Resistance Pattern in Karachi.

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Abstract

Introduction: Cholera is an acute infectious disease of small intestine, caused by the bacterium *Vibrio cholerae*. More than 200 serogroups of *V. cholerae* have been identified. In Iran, Inaba strains were 75% resistant against trimethoprim-sulfamethoxazole in 2011, while during 2012-13 it was 100%. Ogawa strains showed 96% resistance against trimethoprim-sulfamethoxazole in 2011, whereas 100% resistant in 2012, while no resistance was seen in 2013 against the same antibiotic.

Objective: To determine the frequency of vibrio cholera 01 EL TOR (Inaba And Ogawa) and its resistance pattern in Karachi.

Methodology: Samples were collected from patients with acute diarrhea with rice-water stool from Medical Unit, JPMC and NICH, Karachi between October 2015 to August 2016. These samples were then transported to Microbiology Department, Basic Medical Sciences Institute, JPMC, Karachi and processed according to standard protocol.

Results: No growth was noticed in 147 (66.81%) samples while a total of 28 (12.7%) were V. cholerae species, and 45 (20.45%) were other organisms. The distribution of V. cholerae serotypes, out of 28 V. cholerae species, 13 (46.4%) were of Inaba serotypes, 09 (32.1%) were of Ogawa serotypes while 06 (21.4%) were of Non-01 serotypes.

Conclusion: The susceptibility results of tetracycline and trimethoprim-sulfamethoxazole are not very favorable. Involvement of multidrug resistant *V. cholerae* O1 serotypes in the community is a very serious public health concern. Such patients were observed to be very difficult to treat in the community.

Keywords: cholera, Pakistan, Ogawa, trimethoprim-sulfamethoxazole, tetracycline.

Introduction:

Cholera is an acute infectious disease of small intestine, caused by the bacterium *Vibrio cholerae*.[1]The other bacterial isolates which mainly cause diarrhea are Salmonella, Shigella, Campylobacter, and diarrheagenic *E. coli*[2].*V. cholerae* is found in aquatic environment[3] and the cholera disease is regularly found in the poor agricultural countries of Asia, South America and Africa.[4] pandemic started in Indonesia in 1961, then to West Africa in 1970 and then in America in 1991.[5] Pioneer work was done by Snow in 1800s by giving a conclusion that cholera disease spreads from contaminated water.[6] *V. cholerae* is a gram-negative curved rod belonging to the family Vibrionaceae[7]. This bacterium also has some characteristic similarities to the family, Enterobacteriaceae[8]. They are motile and most of them having

single polar flagellum.[9] More than 200 serogroups of *V. cholerae* have been identified[10]. However, only *V. cholerae* O1 serogroup is associated with epidemic and pandemic cholera. *V. cholerae* non-O1 may be associated with severe diarrhea, but do not possess the epidemic potential like *V. cholerae* O1 isolates. The biotype of *V. cholerae* O1 are Classical and El Tor, the serotype of *V. cholerae* O1 El Tor are Inaba, Ogawa and Hikojima. There is no serotype associated with classical biotype of *V. cholerae* O1[11]. Classical biotype of *V. cholerae* O1 was replaced by El Tor biotype in the 7th pandemic.[12] The variant of *V. cholerae* El Tor possesses cholera toxin of classical biotype and currently prevalent in the world.[13]The recent outbreaks of cholera in the world have been due to serogroup *V. cholerae* O1 of El Tor biotype.[14] The rapid emergence and spread of multidrug resistant strains of *V. cholerae* with resulting outbreaks around the world can undermine the success of antimicrobial therapy. There is a great variation in the patterns of antibiotic resistance at different times and different places of the world with multiple antibiotic-resistant *V. cholerae* strains commonly found during epidemics. There are many reports of *V. cholerae* strains showing resistance against tetracycline and fluoroquinolones. A study at the Democratic Republic of Congo (DRC) about resistance pattern of *V. cholerae* described that initially there was resistance against trimethoprim-sulfamethoxazole, which was followed by resistance to nalidixic acid, erythromycin, and chloramphenicol in early 2000s. The strains were susceptible to fluoroquinolones but resistance to tetracycline and ampicillin were also seen during the period between 2007 to 2010. In Iran, Inaba strains were 75% resistant against trimethoprim-sulfamethoxazole in 2011, while during 2012-13 it was 100%. Ogawa strains showed 96% resistance against trimethoprim-sulfamethoxazole in 2011, whereas 100% resistant in 2012, while no resistance was seen in 2013 against the same antibiotic. Kansakar et al in 2010 from Kathmandu found that *V. cholerae* O1 Ogawa biotype El Tor strains were 100% susceptible to tetracycline and ciprofloxacin, while all were resistant to nalidixic acid. Continuous monitoring is required to trace changes in susceptibility patterns and the emergence of resistance to new agents. The study conducted during the period of 2000-2012 in Dhaka 18% of total *V. cholerae O1* strains were MDR [15]. The reports of total cases and deaths during the period of 1947 to 1987 for cholera in Pakistan is shown in table 2. It is clearly showing that the number of cases and deaths were very high from 1947 to 1971. In 2010 during Monsoon season in Pakistan, there were record breaking rains and flood resulted in epidemic of cholera by *V. cholerae O1* of El Tor biotype[16].

Methodology:

A Total of 220 samples were collected from patients with acute diarrhea and rice water stool. Sample size was calculated by Open Epi software. The reference study is "Genomic Epidemiology of *V. cholerae O1* Associated with Floods Pakistan, 2014".

Samples were collected from patients with acute diarrhea and rice-water stool from Medical Unit, JPMC and NICH, Karachi between October 2015 to August 2016. These samples were then transported to Microbiology Department, BMSI, JPMC, Karachi and processed according to standard protocol.

Results:

A total of 220 rice-water stool samples were processed, out of which adult patients (≥14 years) were 88 (40%), including 30 (13.63%) males and 58 (26.36%) females (P<0.09) which is statistically insignificant. Total children (<14 years) were 132 (60%), of which males were 60 (27.27%) and females were 72 (32.72%) (P<0.09) which is statistically insignificant (P=0.09).

The distribution of organisms isolated from the 220 samples processed. No growth was noticed in 147 (66.81%) samples while a total of 28 (12.7%) were V. cholerae Species, and 45 (20.45%) were other organisms.

The distribution of V. cholerae serotypes, out of 28 V. cholerae species, 13 (46.4%) were of Inaba serotypes, 09 (32.1%) were of Ogawa serotypes while 06 (21.4%) were of Non-01 serotypes.

Table No.1: Distribution of organisms according to species on the basis of serology (n=220)

Species	Frequency	Percent
No growth	147	66.81
Vibrio Species	28	12.7
Others	45	20.45
Total	220	100.0

Table No.2: Distribution of vibrio cholerae serotypes (n=28)

Vibrio Species	Frequency	Percent
Inaba	13	46.4
Ogawa	09	32.1
Non-01	06	21.4
Total	28	100.0

Fig No. 1: Age and Gender distribution of subjects

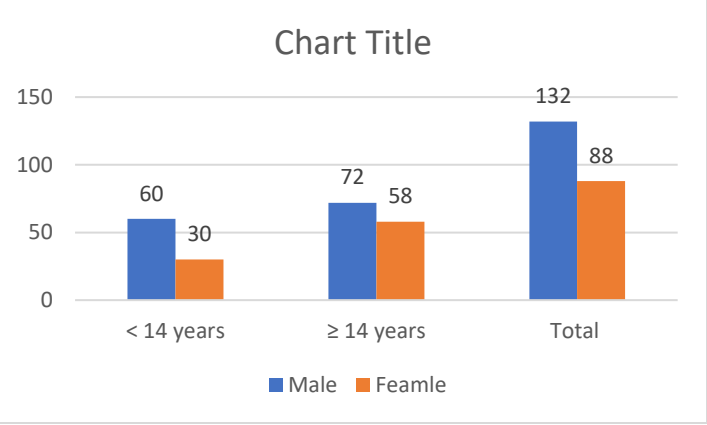


Table No.3: Physical and Microscopic variables of stool samples.

Parameters	Inaba (n=13)	Ogawa (n=9)	V. *Cholera* (Non-01) (n=6)	Others (n=192)
Physical variables				
Color	3 (23.07%)	2 (22.22%)	1 (16.66%)	60 (31.25%)
Blood	10 (76.92%)	1 (11.11%)	1 (16.66%)	60 (31.25%)
Mucus				
pH				
Acidic	3 (23.07%)	1 (11.11%)	1 (16.66%)	82 (42.70%)
Alkaline	10 (76.92)	8 (88.88%)	5 (83.33%)	110 (57.29%)
Microscopic				
No. of RBC/HPF				
0				132 (68.75%)
1-10	10(76.92%)	7 (77.77%)	5 (83.33%)	29 (15.10%)
10-30	3 (23.07%)	2 (22.22%)	1 (16.66%)	21 (10.93%)
> 30				10 (5.20%)
WBC/HPF				
0	5 (38.46%)	3 (33.33%)	1 (16.66%)	100 (52.08%)
1-10	4 (30.76%)	4 (44.44%)	3 (49.66%)	60 (31.25%)
10-30	4 (30.76 %)	2 (22.22%)	2 (33.33%)	20 (10.41%)
> 30				12 (6.25%)
Fat				
Seen	9 (69.23%)	6 (66.66%)	5 (83.33%)	117 (60.93%)
Not seen	4 (30.76%)	3 (33.33%)	1 (16.66%)	75 (39.06%)

Table No.4: Distribution of organisms according to species on the bases of serology (n=220)

Species	Frequency	Percent
No growth	147	66.81
Vibrio Species	28	12.7
Others	45	20.45
Total	220	100.0

Table No. 5: Distribution of vibrio cholerae serotypes (n=28)

Vibrio Species	Frequency	Percent
Inaba	13	46.4
Ogawa	09	32.1
Non-01	06	21.4
Total	28	100.0

Table No.6: Season wise distribution of v. cholerae o1 el tor (Inaba and Ogawa) serotypes and v. cholerae non-01 strains.

Season	Inaba (n=13)	Ogawa (n=9)	V. cholera non-01 (n=6)
Spring (January -April)	1 (7.69%)	0 (%)	0 (%)
Summer (May-June)	4 (30.76%)	4 (44.44%)	3 (50%)
Monsoon (July-August)	6 (46.15%)	3 (33.33%)	3 (50%)
Autumn (September –October)	2 (15.38%)	2 (22.22%)	0 (0%)
Winter (November – December)	0 (%)	0 (%)	0 (0%)

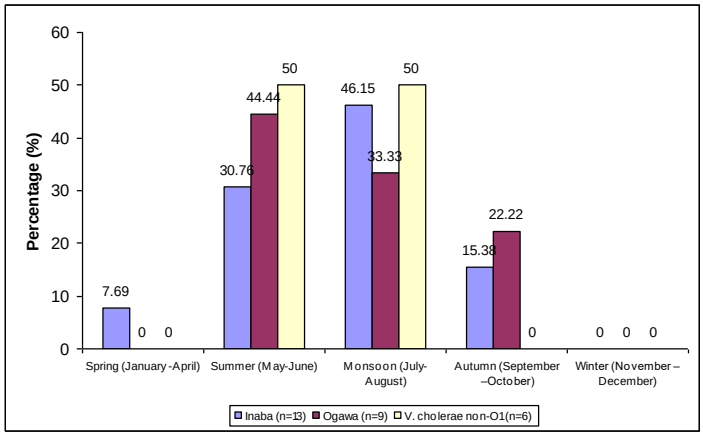
Table No. 7: Sensitivity results of *v. cholerae*01 el tor (Inaba and Ogawa) in both adults (male and female) and children.

Patient Group	Antibiotic		*V. cholerae*01 El Tor Inaba (n=13)	*V. cholerae*01 El TorOgawa (n=9)
Adults ≥ 14 years	AMP	S	4 (100%)	2 (66.66%)
		R	0 (0%)	1 (33.33%)
	CN	S	4 (100%)	3 (100%)
		R	0 (0%)	0 (0%)
	OFX	S	3 (75%)	3 (100%)
		R	1 (25%)	0 (0%)
	SXT	S	2 (50%)	1 (33.33%)
		R	2 (50%)	2 (66.66%)
	TET	S	2(50%)	2 (66.66%)
		R	2 (50%)	1 (33.33%)
Children <14 Years	AMP	S	9 (100 %)	6 (100%)
		R	0 (0%)	0 (0%)
	CN	S	9 (100%)	6 (100%)
		R	0 (0%)	0 (0%)
	OFX	S	9 (100%)	6 (100%)
		R	0 (0%)	0 (0%)
	SXT	S	3 (33.33%)	2 (33.33%)
		R	6 (66.66%)	4 (66.66%)
	TET	S	3 (33.33%)	2 (33.33%)
		R	6 (66.66%)	4 (66.66%)

Table No. 8: Multidrug resistance of *v. cholerae o1* (Inaba and Ogawa) serotypes

Patient Category	Antibiotic	Sensitivity	Inaba (n=13)	Ogawa (n=9)
Adults >14 Years	OFX/TET/SXT	Resistant	1 (25%)	0 (0%)
	AMP/SXT/TET	Resistant	0 (0 %)	1 (33.33%)

Fig No. 2: Season wise distribution of *v. cholerae o1* el tor (Inaba and Ogawa) serotypes and *v. cholerae* non-01 strains



Discussion:

Cholera is endemic in Pakistan but has not been considered as a significant cause of diarrhea. The reporting of cholera is very poor. From 1993 to 2005 and again in 2015, Pakistan has not reported any case of cholera to WHO. It was first time considered as a major cause of diarrhea in 1971.[17] Diarrheal diseases are the leading causes of morbidity and mortality in children particularly in developing countries like Pakistan[18]. It is spread by faeco-oral route following the ingestion of faecally contaminated food or water, person to person transmission or direct contact with infected faeces.

In the present study, cholera cases are noted to increase in summer and monsoon seasons in Pakistan. This finding is inconsistent with different studies conducted in South east Asia[19].Poor standards of environmental hygiene and sanitation and difficulty to get safe drinking water are the key factors for the spread of cholera.

In our study, out of the total isolates there were 13 (46.4%) cases of Inaba serotypes while 9 (32.1%) cases were Ogawa serotypes. However, in contrast to our study there was no Inaba serotype isolated in different studies of Southeast Asia[20]. The prevalence of *V. cholerae* serotypes is different in different regions of the world. In Iraq, all isolates were Inaba serotypes. Inaba serotype was restricted to western parts while Ogawa serotype was prevalent in east and south of the country. In Congo, Ogawa serotype was predominant from 2001 to 2010 but Inaba serotype became predominant in the period from 2011 to 2012[21]. In Pakistan, Inaba serotype surpassed Ogawa in 2005 which was the first report of switching over from Ogawa to Inaba.

In our study, the frequency of Inaba serotype in adults is lower i.e., 4 (30.76%) cases, then that in children i.e., 9 (69.23%) cases and the p-value is not significant (P= 0.7). This is not in agreement with observation of Jameel et al. (2016), who has isolated higher frequency (85%) of Inaba serotype in adult patients. The present study also shows that the frequency of Ogawa serotypes in adults is lower 3 (33.33%) than the frequency in children6 (66.66%). This is consistent with a study done in India during 2016 that reported higher frequency (74%) of Ogawa serotypes in adult patients. In our study cholera cases were mostly identified in children which is in agreement with a published study.[22]

In our study, the higher frequency of Inaba serotype 9 (69.23%) was seen in female patients. In contrast, frequency of Inaba serotypes was higher (55.15%) in male patients in a study conducted in Iraq.[23] In our study 9 (55.55 %) cases of the Ogawa serotype were a male patient which is not in agreement to observation of Gupta et al[24] who has reported higher frequency (58.07%) of Ogawa serotypes in female patients.

Our study shows that all Inaba serotypes isolated in adults and children as well as Ogawa serotypes isolated in children were sensitive to ampicillin while on the other hand out of a total 3 (33.33%) of the isolated Ogawa serotype were resistant to ampicillin. In Indian Punjab, the isolates of Inaba and Ogawa serotypes were found mostly resistant to ampicillin.[25] Ogawa serotypes were found 100% resistant to ampicillin by Gupta et al.[24] In a study at Pano Aqil, Sindh Pakistan[26] there were 37.5% strains of Ogawa serotypes isolated that were resistant to ampicillin. In our study however all isolates (Inaba and Ogawa) were (100%) sensitive to gentamicin in both adults and children; this is agreement with the finding of Mala et al[27] however, Ukaji et al[28] has reported 15.9% resistance to gentamicin, which is in contrast to our findings.

All Ogawa serotypes were 100% susceptible to ofloxacin in both groups. In adult patients infected with Inaba serotype, 25% isolates were resistant, whereas 100% were susceptible in children to ofloxacin. These findings were similar to the study of Mala et al in where isolates were highly susceptible to fluoroquinolones. On the contrary another study in India[19] showed that only 32.90% cases of *V. cholerae*O1 (Inaba and Ogawa) serotypes were sensitive to ofloxacin.

In our results, antibiotic susceptibility pattern shows higher rate of resistance to trimethoprim-sulfamethoxazole and tetracycline in both children and adults. In different studies of the world, resistance to tetracycline and trimethoprim-sulfamethoxazole has been reported worldwide[24]. In the present study, sensitivity of Inaba serotype to tetracyclines isolated in adult and children were 50% and 33.33% respectively. In contrast, most of the *V. cholerae*01 isolates were sensitive to tetracycline (95.3%) in 2009 in India reported all isolated strains of Inaba serotypes being resistant to tetracycline.

In our study, 66.56% of Ogawa serotype cases were sensitive to tetracycline in adults, while in children, 33.33 Ogawa serotypes isolated were sensitive to the same drug. In different studies in South Asia, it was observed that tetracycline was a very effective drug for the treatment of cholera but this drug then gradually lost its efficacy with the passage of time in India[25].

In this study, 50% Inaba serotype were resistant to trimethoprim-sulfamethoxazole in adults while in children, the resistance was 66.66% to same drug. These results are in agreement with the study conducted in India.[26]

In our study, Ogawa serotype in adults while both Inaba and Ogawa serotypes in children were resistant (66.66%) to

trimethoprim-sulfamethoxazole. This is in agreement with published study[29] that reported (75%) resistant strains of Ogawa serotype to trimethoprim-sulfamethoxazole. Our results are not in agreement with the study conducted by Hajia et al[30] who reported no resistant strains of Ogawa serotypes to trimethoprim-sulfamethoxazole in 2013. In Kolkata, India, *V. cholerae*isolates showed emergence of resistance to trimethoprim-sulfamethoxazole from 1980-1990 while tetracycline resistance emerged from 1999-2000[31].

Multidrug resistant strains of *V. cholerae O1* have been found worldwide. Shrestha[32] reported all multidrug resistant *V. cholerae O1* strains in his study in 2015.In our results, multidrug resistant strains of both serotypes were found in adult patients only.

Conclusion:

Our results displayed that the rates of cholera infection were high in monsoon seasons. This may be due to improper water and sanitation facilities and unhygienic practices similar to other developing countries. Susceptibility Results of Tetracycline and trimethoprim-sulfamethoxazole are not very favorable so these should not be considered as the drugs of first choice against both serotypes.

Involvement of multidrug resistant *V. cholerae* O1 serotypes in the community is a very serious public health concern. Such patients were observed to be very difficult to treat in the community.

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References:

1. Altaf Hussain, Nisar Ahmad, Iqbal Ahmed Qarshi, Muhammad Rashid, Zabta K. Shinwari et al. Inhibitory potential of nine mentha species against pathogenic bacterial strains. Pak. J. Bot.2015; 47(6): 2427-2433.
2. Trivedi M, Panchal P. Prevalence of shigella, salmonella and vibrio isolated from stool samples in district hospital. Int J Res med Sci. 2016; 4(9):3884-3886.
3. Megli CJ, Yuen AS, Kolappan S, Richardson MR, Dharmasena MN et al. Crystal structure of the Vibrio cholerae colonization factor TcpF and identification of a functional immunogenic site. J Mol Biol. 2011; 3;409(2):146-58.
4. Klinzing DC, Choi SY, Hasan NA, Matias RR, Tayag E et al. Hybrid *V. cholerae* El Tor lacking SXT identified as the cause of a cholera outbreak in the Philippines. mBio.2015;6(2):e00047-15. doi:10.1128/mBio.00047-15
5. Shapiro RL, Otieno MR, Adcock PM, Phillips-Howard PA, Hawley WA et al. Transmission of epidemic Vibrio cholerae O1 in rural western Kenya associated with drinking water from Lake Victoria: an environmental reservoir for cholera? Am J Trop Med Hyg. 1999;60(2):271-6.
6. Pascual M, Koelle K, Dobson AP. Hyperinfectivity in cholera: a new mechanism for an old epidemiological model? PLoS Med. 2006 Jun;3(6):e280.
7. Al-Sahlany STG. Effect of Mentha piperita essential oil against Vibrio species isolated from local cheeses. Pak J Food Sci. 2016; 26(2):65-71.
8. Winkle S, Refai M, Rohde R. On the antigenic relationship of "Vibrio cholerae" to "Enterobacteriaceae". Ann Inst Pasteur (Paris). 1972 Dec;123(6):775-81.
9. Mylea A. Echazarreta, Karl E. Klose. *Vibrio* Flagellar Synthesis. Front. Cell. Infect. Microbiol., 01 May 2019. https://doi.org/10.3389/fcimb.2019.00131
10. Son MS, Megli CJ, Kovacikova G, Qadri F,Taylor RK. Characterization of Vibrio cholerae O1 E1 Tor biotype variant clinical isolates from Bangladesh and Haiti, including a molecular genetic analysis of virulence genes. J Clin microbiol. 2011; 49(11):3739-3749.
11. Global handwashing partnership. https://globalhandwashing.org/resources/cholera-and-handwashing-fact-sheet/
12. Mutreja A, Kim DW, Thomson NR, Connor TR, Lee JH et al. Evidence for several waves of global transmission in the seventh cholera pandemic. Nature. 2011 Aug 24;477(7365):462-5. doi: 10.1038/nature10392.
13. Amin Marashi SM, Rajabnia R, Imani Fooladi AA, Hojati Z, Moghim S et al. Determination of ctxAB expression in Vibrio cholerae Classical and El Tor strains using Real-Time PCR. Int J Mol Cell Med. 2013 Winter;2(1):9-13.
14. Pal BB, Khuntia HK, Samal SK, Kar SK, Patnaik B. Epidemics of severe cholera caused by El Tor Vibrio cholerae O1 Ogawa possessing the ctxB gene of the classical biotype in Orissa, India. Int J Infect Dis. 2010 May;14(5):e384-9.
15. Kansakar P, Baral P, Malla S, Ghimire GR. Antimicrobial susceptibilities of enteric bacterial pathogens isolated in Kathmandu, Nepal, during 2002-2004. J Infect Dev Ctries. 2011 Mar 21;5(3):163-8.
16. Shah MA, Mutreja A, Thomson N, Baker S, Parkhill J et al. Genomic epidemiology of Vibrio cholerae O1 associated with floods, Pakistan, 2010. Emerg Infect Dis. 2014 Jan;20(1):13-20. doi: 10.3201/.eid2001.130428.
17. Naseer M, Jamali T. Epidemiology, determinants and dynamics of cholera in Pakistan: gaps and prospects for future research. J Coll Physicians Surg Pak. 2014 Nov;24(11):855-60.
18. Memon IA, Murtaza G. Drug sensitivity pattern of cholera in children. J Pak Med Assoc. 2002;52(8):347-8.
19. Kulkarni S and Chillarge C. Antibiotic susceptibility pattern of Vibrio cholerae causing diarrhoea outbreaks in Bidar, North Karnataka, India. Int J Curr Microbiol App Sci. 2015; 4(9):957-961.

20. Karlsson SL, Thomson N, Mutreja A, Connor T, Sur D, Ali M et al. Retrospective Analysis of Serotype Switching of Vibrio cholerae O1 in a Cholera Endemic Region Shows It Is a Non-random Process. PLoS Negl Trop Dis. 2016 5;10(10)
21. Miwanda B, Moore S, Muyembe JJ, Nguefack-Tsague G, Kabangwa IK et al. Antimicrobial Drug Resistance of Vibrio cholerae, Democratic Republic of the Congo. Emerg Infect Dis. 2015;21(5):847-51.
22. Hashizume M, Armstrong B, Hajat S, Wagatsuma Y, Faruque et al. The effect of rainfall on the incidence of cholera in Bangladesh. Epidemiology 2008; 19(1):103-10.
23. Hussain AM, Lafta RK. Trend of cholera in Iraq in the time of unrest. Mustansiriya Med J. 2019;18(1):1-4.
24. Gupta PK, Pant ND, Bhandari R, Shrestha P. Cholera outbreak caused by drug resistant Vibrio cholerae serogroup O1 biotype ElTor serotype Ogawa in Nepal; a cross-sectional study. Antimicrob Resist Infect Control. 2016; 4;5:23. doi: 10.1186/s13756-016-0122-7.
25. Kumar A, Oberoi A. Vibrio isolates from cases of acute diarrhea and their antibiotic susceptibility pattern in a tertiary care hospital of Punjab. CHRISMED J Health Res 2014;1:254-7.
26. Lodhi M, Munir T, Karamat KA. Dehydrating diarrhoea in children due to vibrio cholerae. Park Arm Forces Med J 2006; 56(1).
27. Mala E, Oberoi A and Alexander VS. Vibrio isolates from cases of acute diarrhea and their antimicrobial susceptibility pattern in a tertiary care hospital. Int J Basic App Sci. 2014; 3(1):35-37.
28. Ukaji DC, Kemajou TS, Ajugwo AO, Ezeiruaku FC and Eze EM. Antibiotic susceptibility patterns of Vibrio cholera O1 isolated during cholera outbreak in Uzebba (Edo State). Open Sci J Bioscience Bioeng. 2015; 2(3):33-36.
29. Kar SK, Pal BB, Khuntia HK, Achary KG, Khuntia CP. Emergence and spread of tetracycline resistant Vibrio cholerae O1 El Tor variant during 2010 cholera epidemic in the tribal areas of Odisha, India. Int J Infect Dis. 2015;33:45-9.
30. Hajia M, Saboorian R and Rahbar M. Antimicrobial resistance patterns of isolated Vibrio cholerae strains. Int J Enteric Pathog. 2016; 4(1):e1719.
31. Ghosh P, Naha A, Pazhani GP, Ramamurthy T, Mukhopadhyay AK, et al. Genetic Traits of Vibrio cholerae O1 Haitian Isolates That Are Absent in Contemporary Strains from Kolkata, India. PLoS ONE. 2014;9(11): e112973.
32. Shrestha UT, Adhikari N, Maharjan R, Banjara MR, Rijal KR et al. Multidrug resistant Vibrio cholerae O1 from clinical and environmental samples in Kathmandu city. BMC Infect Dis 2015; 15:104-110.