PDF version

S. Irfan,[1] N. Fasih,[1] N.K. Ghanchi[1] and E. Khan[1]

O139 **O1**

2012-1999

ABSTRACT In the past decade the importance of non-O1 and non-O139 strains of Vibrio cholerae has been highlighted globally. This study aimed to evaluate the frequency and antimicrobial susceptibility profile of non-O1 and non-O139 V. cholerae in Pakistan. Data of stool specimens yielding growth of non-O1 and non-O139 V. cholerae isolated at a national referral laboratory from 1999 to 2012 were retrospectively analysed and evaluated for resistance to ampicillin, tetracycline, chloramphenicol, co-trimoxazole and ofloxacin. A total of 95 800 stool samples submitted over 1999–2012 yielded 3668 strains of V. cholerae, of which 6% were non-O1 and non-O139 V. cholerae. A high isolation rate was found in the summer season, with a peak in the year 2003. Antimicrobial susceptibility data revealed increasing resistance to co-trimoxazole and ampicillin, but strains remained highly susceptible to ofloxacin. Active surveillance of serotypes and antimicrobial susceptibility is essential to predict future epidemics and define measures to curtail the disease.

Fréquence d’isolation et profil de sensibilité de Vibrio cholerae non-O1 et

non-O139 dans un laboratoire de soins de santé tertiaires, 1999-2012

RÉSUMÉ Au cours des dix dernières années, l'importance des souches de Vibrio cholerae non-O1 et non-O139 a été mise en avant à l'échelle mondiale. La présente étude visait à évaluer la fréquence de l'isolation des souches de Vibrio cholerae non-O1 et non-O139 et leur profil de sensibilité aux antimicrobiens au Pakistan. Les données d'échantillons de selles ayant permis la croissance de V. cholerae non-O1 et non-O139 isolés dans un laboratoire national spécialisé entre 1999 et 2012 ont été analysées et évaluées rétrospectivement pour leur résistance à l'ampicilline, la tétracycline, au chloramphénicol, au co-trimoxazole et à l'ofloxacine. Au total, 95 800 échantillons de selles soumis entre 1999 et 2012 ont produit 3668 souches de V. cholerae, parmi lesquelles 6 % étaient des souches de V. cholerae non-O1 et non-O139. Un taux d'isolation élevé a été observé au cours de l'été, avec un pic en 2003. Les données relatives à la sensibilité aux antimicrobiens ont révélé une augmentation de la résistance au co-trimoxazole et à l'ampicilline, mais les souches restaient fortement sensibles à l'ofloxacine. Une surveillance active des sérotypes et de la sensibilité aux antimicrobiens est essentielle pour prévenir les épidémies et définir des mesures permettant d'enrayer la maladie.

[1]Department of Pathology and Microbiology, Aga Khan University Hospital, Karachi, Pakistan (Correspondence to S. Irfan: seema.irfan@aku.edu).

Received: 22/07/14; accepted: 24/11/15

Introduction

Food- and water-borne illnesses are leading public health problems around the world. Although in developed countries the predominant bacterial causes of diarrhoea include pathogens such as Salmonella and Campylobacter spp. (1), in developing countries Vibrio cholerae still remains the top cause of watery diarrhoea (2). V. cholerae is classified to over 200 serotypes on the basis of variations in the lipopolysaccharide somatic antigen (3). V. cholerae belonging to serogroups O1 and O139 has been associated with epidemics and pandemics of cholera due to production of cholera toxin (2–5). Other serotypes are collectively known as non-O1 and non-O139 or non-typeable V. cholerae. A majority of these strains are usually responsible for sporadic cases of diarrhoea (6–8). However, the pathogenic potential of non-O1 and non-O139 V. cholerae should not be underestimated as some strains are clearly pathogenic, due either to cholera toxin production or to other virulence factors such as heat-stable enterotoxin, haemolysin, repeats-in-toxin and type 3 secretion systems (7). Occasionally, these strains are responsible for outbreaks of cholera (9–11). According to the United States Centers for Disease

Control and Prevention (CDC), non-O1 and non-O139 V. cholerae are the third most commonly reported group of Vibrio spp., and since the year 2000 they accounted for around 40 cases of diarrhoea per year reported to the CDC (12). Moreover, in immunocompromised people these bacteria have been associated with a severe form of disease that may lead to sepsis and other life-threatening conditions (13–15). The susceptibility pattern of these reported isolates are diverse, showing a varying prevalence of different serovars in different regions.

In Pakistan V. cholerae O1 is one of the commonest organisms responsible for diarrhoeal outbreaks (16). An outbreak of O139 was also reported in 2002 (5). However, data on the isolation rate and antibiotic susceptibility pattern of non-O1 and non-O139 V. cholerae are lacking. Such data are valuable for the empirical treatment of patients in special circumstances and to compare with reports from other countries. This study therefore aimed to evaluate the frequency and antimicrobial profile of non-O1 and non-O139 V. cholerae in Pakistan from stool samples over the years 1999–2012.

Method

Study design and setting

This was a retrospective analysis of antimicrobial resistance among non-O1 and non-O139 V. cholerae strains isolated at the clinical microbiology laboratory of Aga Khan University hospital in Karachi, Pakistan, for diagnostic purposes. This laboratory caters for inpatients as well as outside referrals from other hospitals, clinics and general practitioners across the country. All 95 800 stool samples submitted to the laboratory for analysis from the years 1999 to 2012 were included in the study.

The study protocol and consent procedures were approved by the ethics review committee of the Aga Khan University Hospital. Specific verbal or written consent from patients was not required as the data used were obtained from laboratory records and used anonymously.

Data collection

All stool samples for culture were processed according to standard practice and were inoculated on the following media: MacConkey, Salmonella-Shigella, Campylobacter and tellurite taurocholate gelatin agar (TTGA) (Oxoid). Stool samples were also inoculated in selenite F broth and alkaline-peptone water and sub-cultured on solid media after overnight incubation. Suspected colonies from TTGA were biochemically confirmed using analytical profile index 20 Enterobacteriaceae (API 20 E) (BioMerieux). V. cholerae O1 serogroups were performed using polyvalent O1 antisera, for Ogawa and Inaba strains (Murex Diagnostic) and serogroup O139 (Dienka Sieken). Non-O1 and non-O139 V. cholerae were reported when an isolate had a biochemical profile (using API 20 E) of V. cholerae but failed to agglutinate by V. cholerae

antisera O1 and O139.

Antimicrobial drug susceptibility assay was performed by the disk diffusion method using commercially available disks (Becton Dickinson; Sparks Glencoe). The antibiotics tested were: ampicillin (10 µg), tetracycline (30 µg), co-trimoxazole (1.25/23.75 µg), chloramphenicol (30 µg) and ofloxacin (5 µg). Since there were no interpretive breakpoints for V. cholerae available in the Clinical and Laboratory Standard Institute guidelines, breakpoints for Enterobacteriaceae were adopted for V. cholerae (17). Escherichia coli (ATCC 25922) strain was used as a quality control.

Data management and statistical analysis

Data extracted from the computerized information system of the hospital were transferred to the statistical software SPSS, version 19.0. Frequencies with percentages for age, seasonal distribution and isolation rate were analysed. The resistance pattern of non-O1 and non-O139 V. cholerae was evaluated among different age groups.

Results

During the study period (1999–2012), a total of 95 800 stool samples for culture and susceptibility testing were processed at the Aga Khan University Hospital laboratory. The yield of positive samples (isolation of at least 1 diarrhoea-causing bacterial pathogen including Salmonella spp., Shigella spp., V. cholerae, Campylobacter spp. and Aeromonas spp.) was 20 125 (21%). Out of these positive cultures 3668 isolates (18%) were V. cholerae group (Table 1). Throughout the study period (1999–2012) V. cholerae O1 serogroups remained the predominant strain but a changing trend from serotype Ogawa to Inaba was seen in the years 2005–6 and 2012 respectively and again from Inaba to Ogawa was seen in 2007 (Table 1). V. cholerae O139 serogroup emerged only in the years 2000–2002.

Of the total V. cholerae isolates, 233 (6%) failed to show agglutination by O1 (Ogawa, Inaba) and O139 antisera. These isolates were confirmed as V. cholerae by API 20E, and reported as non-O1 and non-O139 V. cholerae. Table 1 shows the annual rate of isolation of non-O1 and non-O139 V. cholerae over the study period; the highest number of cases were seen in the year 2003.

The majority of stool cultures yielding non-O1 and non-O139 V. cholerae (98%) were submitted from different locations of Karachi city. From other locations 1% were isolated from Hyderabad and 0.7% and 0.3% were from Mir Pur Khas and Jacobad respectively. Demographic data showed that 122 cases (52%) of non-O1 and non-O139 V. cholerae were in children aged ≤ 14 years and 111 (48%) in those aged > 14 years old, and more cases were in males (135, 58%) than females (98, 42%). Seasonal variations in isolation frequency were observed, with the few or no isolates during winter and a maximum rate during summer with peaks from May to August each year (Figure 1).

The annual trend of antimicrobial susceptibility data revealed increasing resistance to antibiotics such as co-trimoxazole and ampicillin. The lowest rate of resistance was observed against ofloxacin, followed by tetracycline and chloramphenicol, as shown in Figure 2 . Table 2 shows that for most antibiotics antimicrobial resistance rates were similar between children and older age groups, except for co-trimoxazole which showed higher resistance among children aged ≤ 14 years than adolescent/adults aged > 14 years (52% versus 33%).

Discussion

To the best of our knowledge this is the first study reporting the frequency of non-O1 and non-O139 V. cholerae from clinical samples tested at a diagnostic laboratory of Pakistan. We found that the rate of non-O1 and non-O139 V. cholerae remained at a low level (≤ 10%) of the total V. cholerae group throughout the study period except from 2002 to 2004 where an upsurge was observed from 13% to 27%, with a prominent surge in the year 2003.

Our findings of a low isolation rate of non-O1 and non-O139 V. cholerae from clinical samples (overall 6% of total V. cholerae) is consistent with the sporadic form of diarrhoea caused by endemic strains of non-O1 and non-O139 V. cholerae reported from other countries in the region. A literature search showed Indian studies reporting variable isolation frequencies of non-O1 and non-O139 V. cholerae from clinical samples. For example, 4% and 5% isolation rates of non-O1 and non-O139 V. cholerae were reported from Sevagram in Maharashtra state and Delhi respectively (18,19), and higher isolation rates of 13% and 24% from Kolkata and Hubli respectively (17,20). Similarly, reports from Thailand showed a lower rate of up to 3% of non-O1 and non-O139 V. cholerae among V. cholerae isolates (21).

The increase in frequency of non-O1 and non-O139 V. cholerae noted in 2003 is suggestive of a possible outbreak in that year. Non-O1 and non-O139 V. cholerae strains detected during that period also had unique features of co-trimoxazole resistance of 100% compared with a baseline of 35–40% (Figure 2). A plausible association of this high isolation rate could be related to heavy rainfalls leading to nationwide flooding in 2003. These findings are suggestive of a

possible emergence of a novel strain with a unique antibiogram, and perhaps increased pathogenicity, leading to symptomatic disease. Due to the retrospective nature of the study we were not been able to perform molecular strain typing and could not retrieve clinical data to confirm the outbreak situation with the novel strain.

Interestingly a majority of non-O1 and non-O139 V. cholerae strains were isolated from stool samples received from Karachi, a coastal city which has a questionable supply of clean drinking water and high rates of enteric fever and other gastrointestinal infections in the community (22,23). Additionally a lack of proper surveillance of diarrhoeal outbreaks leads to paucity of data. Similar upsurges in the isolation rate of non-O1 and non-O139 V. cholerae have been reported from India during the period 2002–04, showing replacement of predominant V. cholerae O1 serotypes (20). These findings suggest the possibility of a regional outbreak with novel serotypes and emphasizes the need for continuous surveillance and monitoring of regional diarrhoeal strains.

Throughout the duration of the current study (1999–2012) V. cholerae O1 serogroups remained the predominant serotype. Another interesting finding of our study is related to V. cholerae O139. It seems that a new serotype with a different antibiogram (5) had emerged in the year 2000–02. This is comparable with the similar trend reported from India (19). Further comparison of previously published antimicrobial susceptibility data of V. cholerae O139 from Pakistan (5) is comparable with India (19) and strains from both regions had a similar susceptibility pattern to reported antimicrobials including co-trimoxazole (5,19). This again highlights the likelihood of circulation of particular type of V. cholerae O139 in the Indian subcontinent during that period. Non-O1 and non-O139 V. cholerae were likely to be the origin of the new virulence gene, as emergence of the O139 serovar resulted due to horizontal gene transfer between V. cholerae O1 and O22 (24,25). Therefore, continuous monitoring of these serovars will provide valuable information on mechanisms of virulence.

Finally, the current data showed a fluctuating pattern of susceptibility from year to year of non-O1 and non-O139 V. cholerae isolates to the antibiotics commonly used against cholera. Low resistance to tetracycline was seen except in the year 2012. Resistance to co-trimoxazole peaked in 2003 and 2012. Therefore with empirical use of World Health Organization recommended drugs such as tetracycline and doxycycline intended for treatment of cholera, continuous monitoring of antimicrobial resistance surveillance should be maintained.

Conclusion

In conclusion, this study found that, despite annual fluctuations, the rate of non-O1 and non-O139 V. cholerae remained a significant cause of diarrhoeal illness in Pakistan, as it is in

neighbouring regions, with the possibility of emergence of novel pathogenic strains. These findings emphasize the need for continuous surveillance of serotypes and monitoring of antimicrobial resistance of regional diarrhoeal strains to predict future epidemics and define measures to curtail the disease. Surveillance data will also provide baseline information to develop empirical antibiotic choice, effective vaccines and preventive strategies.

Acknowledgements

We thank the faculty and staff of the department of clinical microbiology of the Aga Khan University Hospital, Karachi, for their support in this study.

Funding: None.

Competing interests: None declared.

References

1. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, et al. Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis. 2011 Jan;17(1):7–15. PMID:21192848

2. Garg P, Chakraborty S, Basu I, Datta S, Rajendran K, Bhattacharya T, et al. Expanding multiple antibiotic resistance among clinical strains of Vibrio cholerae isolated from 1992–7 in Calcutta, India. Epidemiol Infect. 2000 Jun;124(3):393–9. PMID:10982062

3. Morris JG Jr. Cholera and other types of vibriosis: a story of human pandemics and oysters on the half shell. Clin Infect Dis. 2003 Jul 15;37(2):272–80. PMID:12856219

4. Sundaram SP, Revathi J, Sarkar BL, Bhattacharya SK. Bacteriological profile of cholera in Tamil Nadu (1980–2001). Indian J Med Res. 2002 Dec;116:258–63. PMID:12807153

5. Jabeen K, Hasan R. Re-emergence of Vibrio cholerae O139 in Pakistan: report from a tertiary care hospital. J Pak Med Assoc. 2003 Aug;53(8):335–8. PMID:14558737

6. Dalsgaard A, Forslund A, Bodhidatta L, Serichantalergs O, Pitarangsi C, Pang L, et al. A high proportion of Vibrio cholerae strains isolated from children with diarrhoea in Bangkok, Thailand are multiple antibiotic resistant and belong to heterogenous non-O1, non-O139 O-serotypes. Epidemiol Infect. 1999 Apr;122(2):217–26. PMID:10355785

7. Chatterjee S, Ghosh K, Raychoudhuri A, Chowdhury G, Bhattacharya MK, Mukhopadhyay AK, et al. Incidence, virulence factors, and clonality among clinical strains of non-O1, non-O139 Vibrio cholerae isolates from hospitalized diarrheal patients in Kolkata, India. J Clin Microbiol. 2009 Apr;47(4):1087–95. PMID:19158257

8. Rudra S, Mahajan R, Mathur M, Kathuria K, Talwar V. Cluster of cases of clinical cholera due to Vibrio cholerae 010 in east Delhi. Indian J Med Res. 1996 Feb;103:71–3. PMID:8714141

9. Luo Y, Ye J, Jin D, Ding G, Zhang Z, Mei L, et al. Molecular analysis of non-O1/non-O139 Vibrio cholerae isolated from hospitalised patients in China. BMC Microbiol. 2013;13:52. PMID:23497008

10. Tobin-D'Angelo M, Smith AR, Bulens SN, Thomas S, Hodel M, Izumiya H, et al. Severe diarrhea caused by cholera toxin-producing Vibrio cholerae serogroup O75 infections acquired in the southeastern United States. Clin Infect Dis. 2008 Oct 15;47(8):1035–40. PMID:18781876

11. Bagchi K, Echeverria P, Arthur JD, Sethabutr O, Serichantalergs O, Hoge CW. Epidemic of diarrhea caused by Vibrio cholerae non-O1 that produced heat-stable toxin among Khmers in a camp in Thailand. J Clin Microbiol. 1993 May;31(5):1315–7. PMID:8501234

12. National cholera and vibriosis surveillance. Atlanta (GA): Centers for Disease Controls and Prevention (http://www.cdc.gov/nationalsurveillance/cholera-vibrio-surveillance.html, accessed 13 December 2015).

13. Farina C, Luzzi I, Lorenzi N. Vibrio cholerae O2 sepsis in a patient with AIDS. Eur J Clin Microbiol Infect Dis. 1999 Mar;18(3):203–5. PMID:10357055

14. Strumbelj I, Prelog I, Kotar T, Dovecar D, Petras T, Socan M. A case of Vibrio cholerae non-O1, non-O139 septicaemia in Slovenia, imported from Tunisia, July 2005. Euro Surveill. 2005 Oct;10(10):E051020.6. PMID:16790890

15. Sack DA, Sack RB, Nair GB, Siddique AK. Cholera. Lancet. 2004 Jan 17;363(9404):223–33. PMID:14738797

16. Sheikh A, Khan A, Malik T, Fisher-Hoch SP. Cholera in a developing megacity; Karachi, Pakistan. Epidemiol Infect. 1997 Dec;119(3):287–92. PMID:9440430

17. Dutta D, Chowdhury G, Pazhani GP, Guin S, Dutta S, Ghosh S, et al. Vibrio cholerae non-O1, non-O139 serogroups and cholera-like diarrhea, Kolkata, India. Emerg Infect Dis. 2013 Mar;19(3):464–7. PMID:23622872

18. Narang P, Mendiratta DK, Deotale VS, Narang R. Changing patterns of Vibrio cholerae in Sevagram between 1990 and 2005. Indian J Med Microbiol. 2008 Jan-Mar;26(1):40–4. PMID:18227596

19. Das S, Gupta S. Diversity of Vibrio cholerae strains isolated in Delhi, India, during 1992–2000. J Health Popul Nutr. 2005 Mar;23(1):44–51. PMID:15884751

20. Chandrasekhar MR, Krishna BV, Patil AB. Changing characteristics of Vibrio cholerae: emergence of multidrug resistance and non-O1, non-O139 serogroups. Southeast Asian J Trop Med Public Health. 2008 Nov;39(6):1092–7. PMID:19062701

21. Supawat K, Huttayananont S, Sawanpanyalert P, Aswapokee N, Mootsikapun P. Antimicrobial resistance surveillance of Vibrio cholerae in Thailand from 2000 to 2004. J Med Assoc Thai. 2009 Aug;92 Suppl 4:S82–6. PMID:21294503

22. Jabeen K, Zafar A, Irfan S, Khan E, Mehraj V, Hasan R. Increase in isolation of extended spectrum beta lactamase producing multidrug resistant non typhoidal Salmonellae in Pakistan. BMC Infect Dis. 2010 Apr 22;10:101. PMID:20409348

23. Irfan S, Ahmad A, Guhar D, Mahmood S, Zafar A. Fluoroquinolone and macrolide co-resistance in clinical isolates of Campylobacter species: a 15-year study in Karachi, Pakistan. East Mediterr Health J. 2012 Dec 4;16(12):1226–30. PMID:24988396

24. Li M, Shimada T, Morris JG Jr, Sulakvelidze A, Sozhamannan S. Evidence for the emergence of non-O1 and non-O139 Vibrio cholerae strains with pathogenic potential by exchange of O-antigen biosynthesis regions. Infect Immun. 2002 May;70(5):2441–53. PMID:11953381

25. Dumontier S, Berche P. Vibrio cholerae O22 might be a putative source of exogenous DNA resulting in the emergence of the new strain of Vibrio cholerae O139. FEMS Microbiol Lett. 1998 Jul 1;164(1):91–8. PMID:9675855

Thursday 11th of July 2024 02:21:52 PM