Available online at *www.ujpronline.com*
Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal
ISSN: 2831-5235 (Print); 2456-8058 (Electronic)

RESEARCH ARTICLE

EPIDEMICITY OF *VIBRIO CHOLERA* IN SANA'A CITY, YEMEN: PREVALENCE AND POTENTIAL DETERMINANTS

Ali Abdulkarim Sheiban[1], Hassan A. Al-Shamahy[2], Nashwan M. Alattab[3], Al-Kasem M. A. Abbas[4]
[1]*Department of Medicine, Faculty of Medicine and Health Sciences, Sana'a University, Republic of Yemen.*
[2]*Medical Microbiology and Clinical Immunology, Faculty of Medicine and Health Sciences, Sana'a University.*
[3]*Ministary of health and Population, Sana'a city, Republic of Yemen.*
[4]*Department of Maxillo-Facial Surgery, Faculty of Dentistry, Sana'a University, Republic of Yemen.*

Article Info:

Article History:
Received: 12 October 2017
Reviewed: 8 November 2017
Accepted: 31 December 2017
Published: 15 January 2018

Cite this article:
Sheiban AA, Al-Shamahy HA, Alattab NM, Al-Kasem MAA. Epidemicity of *Vibrio cholera* in sana'a city, Yemen: prevalence and potential determinants. Universal Journal of Pharmaceutical Research 2017; 2(6): 1-5.
http://doi.org/10.22270/ujpr.v2i6.R1

***Address for Correspondence:**
Dr. Hassan A. Al-Shamahy, Faculty of Medicine and Health Sciences, Sana'a University, P.O. Box 775 Sana'a, Yemen,Tel: +967-1-239551, Mobile: +967-770299847.
E-mail: *shmahe@yemen.net.ye*

Abstract

Objectives: In 2017, a total of 889854 suspected cholera cases with 2578 deaths were reported from Yemen, thus WHO considered these figures to be the worst epidemic of cholera in recent history of humanity. The aims of the study were to determine the prevalence of *Vibrio cholera* and protozoa causes in severe diarrhea patients and the potential risk factors of the contracting *Vibrio cholera.*
Methods: Hospital-based diarrhoeal disease surveillance has been done for 12 days in Bany-alharth district of Sana'a city, where all patients admitted with severe diarrhoea in all health centers in the area were enrolled and tested for *Vibrio cholerae*, and others causes. The study was conducted on 345 patients and demographic, clinical, and potential risk factors were collected, then stool specimens were collected and processed by standard methods.
Results: The prevalence of *V. cholerae* was 8.1%, intestinal *Entamoeba histolytica* was 50.7%, and *Giardia lamblia* was 6.7% and one case of *EPEC* while 42% of diarrheal cases were undiagnosed. There was slightly increasing in the rate of *V. cholerae* infection with increasing age (15%). Also there were significant risk factors of dispose sewages to surround environment (OR=3.4 times**,** p=0.02) and reused Jerry can bottles for drinking water (OR=3.1, p=0.03) with *V. cholerae* infection *Vibrio cholera* infection rate and intestinal protozoa infection rates were significantly high.
Conclusion: The findings emphasize that there is cholera epidemic in Sana' city and diarrheal epidemic due to various diagnosed and non diagnosed pathogenic microorganisms which may predispose population of the study to significant health risks.
Keywords: Cholera, diarrhea, prevalence, risk factors, Sana'a city, Saudi Aggression, Yemen.

INTRODUCTION

Cholera occurs following infection of the intestine by the O1 or O139 sero-groups of the bacterium *Vibrio cholerae*[1,2,3,4]. About 20% of infected individuals develop acute, watery diarrhoea and 10–20% of these progress severe watery diarrhoea[5]. Even though case-fatality rates have dropped due to oral and intravenous rehydration therapy, cholera can cause severe disease because of its rapid onset; residents in low-income locations as Yemen are at particularly high risk of infection in areas where public health systems cannot cope with outbreaks as in Yemen in which about 60% of public health system have been destroyed by the Saudi aggression on Yemen for 3 years and still continue[6]. In 2017, a total of 889854 suspected cholera cases were reported from Yemen, including 2578 deaths, to the World Health Organization (WHO)[6,7]. So WHO considered these figures to be worst epidemic of cholera in recent history of humanity. As the fact that the WHO considered reported figures from endemic areas of cholera are underestimates, as poor surveillance systems and fear of negative impact on trade and tourism in many countries likely led to under-reporting[7,8]. WHO estimates that officially reported cases represent only 5–10% of the actual number occurring worldwide annually[6]. Cholera is an endemic in Yemen[9]. In Yemen, cholera occurs year-round with seasonal peaks typically before and after rainy seasons with limited number of cases[9]. The true burden of cholera is unknown in Yemen due to the lack of a population-based surveillance system. The estimation

of cholera prevalence is particularly important to take effective control measures, including the provision of clean water, improved hygiene and sanitation, and introduction of cholera vaccines. Oral cholera vaccines have been found to be safe and effective[10,11,12]. However, modeling studies have shown that water and sanitation measures may provide an equally viable solution, especially in the long term, since the immunization granted by vaccines wanes over time[13,14,15]. Two types of inactivated cholera vaccines are currently available: one containing recombinant cholera toxin B subunit and killed cholera whole cells (rBS-WC) and the other containing only killed cholera whole cells (WC)[16,17]. Field trials demonstrated that both vaccines provided >50% protection for 3 yrs[16,18]. However, the WC vaccine is cheaper, at US$1.85 per dose in the public sector, with a protective efficacy of 66% during the third year of follow-up, as reported in a recent study from Kolkata, India[19]. Credible data regarding incidence of cholera is currently unavailable in Yemen, which limits the validity of any cost-effectiveness evaluation of a potential intervention programme. The aims of the study were to determine the prevalence of *V. cholera* and protozoa causes among Yemeni patients suffering from severe diarrhea and the potential risk factors of the contracting *V. cholera.*

MATERIALS AND METHODS

Case definition

We defined severe diarrhoea as frequent loose or liquid stools for which a person had to be admitted to a healthcare facility, or had to receive intravenous rehydration, or had died as a result of the diarrhoeal illness.

Data collection

Data including demographic data of the patients, clinical information, and potential risk factors as water sources, food ingestion, sewage discarding, *etc.* time of disease, time of collection the specimen, etc. The findings were recorded in a form with laboratory results.

Laboratory testing

Following rectal swab or stool specimens collection, samples were immediately placed in Cary–Blair transport media. All samples were cultured in the Al-Thorah hospital microbiology laboratory using standard bacteriological methods[20,21]. In the laboratory, the rectal swabs or stool specimens were incubated in alkaline peptone water (APW) at 37°C for 4 h. The rectal swabs or stool specimens, as well as the 4-h broth enrichments, were inoculated by streaking on taurocholate-tellurite-gelatin agar (TTGA). Colonies resembling *V. cholerae* were agglutinated with antisera specific for *V. cholerae* O1 and *V. cholerae* O139[21].

Sample size

We calculated the sample size for healthcare utilization survey in the catchment area of surveillance hospitals by using the sample size calculation; it was assumed that in the catchment area of Sana'a city-based surveillance health centers and hospitals there would be about 800 000 severe diarrhoea patients per year. With expected frequency of cholera among them equal to 8.1%, and with acceptable margin of error 2.9%, with design effect 1 and for one cluster, we need at least 340 severe diarrhoeal cases in 95% confidence level.

Cholera case definitions and data analysis

All patients with positive colonies of *V. cholerae* and agglutinated with antisera specific for *V. cholerae* O1 and *V. cholerae* O139 were considered to have had cholera infection. To relate possible risk factors for cholera infection, the data were examined in a case-control study format. For severe diarrhoeal cases with evidence of infection with *V. cholerae* were matched up with those who were *V. cholerae* negative. Differences in categorical variables were assessed using Fisher's exact tests where appropriate. Ninety-five percent confidence intervals for odds ratios were calculated according to the method of Cornfield and 95% confidence limits for simple proportions were calculated by an exact binomial method using EPI-INFO.

Ethical approval

The field team obtained written consent from the identified severe diarrhoeal cases or their guardians. Assent was taken from participants aged between 11 and 17 years. In the surveillance hospitals, consent was also obtained from patients with diarrhoea before collecting the stool specimen. The study protocol was reviewed and approved by the Ethics Committee of Sana'a University, Faculty of Medicine and Health Sciences.

RESULTS

The study includes 345 patients of sever diarrhoea in Sana'a city during a period of 12 days, starting in July 1st 2017 and ending in July 12th 2017. The tested patients ages were ranged from 1 years to 65 years old, most of individuals were in age groups of <5 years (47.8%), followed by age group 5-10 years (43.1%), while only 7.8% of the total were in age group 11-20 years and only 1.5% were in age group ≥ 21 years (Table 1).

Table 1: Age distribution of the patients suffering from severe diarrhoea that tested for *V. cholerae* infection positivity.

Age groups	Total (n =345)	
	No.	%
< 5 years	165	47.8
5 - 10 years	149	43.1
11 - 20 years	27	7.8
≥ 21 years	5	1.5

The prevalence of *V. cholerae* was 8.1%, 3.5% of them as single cause and 4.6% were suffering from co-infection of *V. cholerae* (4.4% with *E. histolytica*). The prevalence of intestinal *E. histolytica* was 50.7%, in which 40.9% of them as single cause and 9.9% were suffering from co-infection with other micro-organisms. The prevalence of intestinal *G. lamblia* was 6.7%, in which 2.6% of them as single cause and 4.1% were suffering from co-infection.

Table 2: The frequency of different bacterial, protozoa and parasites that diagnosed among The patients suffering from severe diarrhoea whom tested for *V. cholerae* infection.

Agents	Frequency	
	Number	Percentage
V. cholerae	28/345	8.1
Single infection	12/345	3.5
Co-infection	16/345	4.6
E. histolytica	175/345	50.7
Single infection	141/345	40.9
Co-infection	34/345	9.9
Giardia lamblia	23/345	6.7
Single infection	9/345	2.6
Co-infection	14/345	4.1
H. nana	7/345	2
Single infection	3/345	0.9
Co-infection	4/345	1.2
EPEC	2/345	0.6
Single infection	2/345	0.6
Co-infection	0/345	
Undiagnosed	145/345	42
Total diagnosed	200/345	58

Table 3: The association between *V. cholerae* infections and the age groups of the patients suffering from severe diarrhoea.

Age groups	*V. cholerae* positive culture (n=28)		OR	CI	χ^2	*p*
	No.	%				
< 5 years, n=162	10	6.2	0.6	0.26-1.3	1.5	0.21
5-10 years, n=149	14	9.4	1.3	0.1-2.9	0.57	0.44
11 -20 years, n=27	4	15	2.1	0.7-6.6	1.7	0.18
≥ 21 years, n=5	0	0	undefined			
Crude rate N=345	28	8.1				

OR- odds ratio => 1 (risk), CI- Confidence intervals 1 to more than 1, X^2- Chi-square => 3.9 (significant), *p* -Probability value =< 0.05 (significant)

However, a low prevalence of *EPEC* (0.6%) and *H. nana* (2%) were very low. On other hand 42% of diarrheal cases were undiagnosed (unknown causes) (Table 2). There was slightly increasing in the rate of *V. cholerae* infection with increasing age, in which the highest rate occurred in age group 11-20 years old (15%), followed by 5-10 years old (9.4%), while the rates in age group < 5 years old was 6.2%, and in ≥ 21 years were zero% (Table 4). When the sources of drinking water versus *V. cholerae* infection were considered, there was a highly significant increasing in the rate of *V. cholerae* infection with Jerry can bottles using (16.1%, with OR=3.1 times, CI=1.5-6.9, and *p*=0.02). However, there was no significant association between *V. cholerae* infections and other sources of drinking water (Table 4). There was a highly significant increasing in the rate of *V. cholerae* infection with dispose sewages to the house surround environment (rate=21%, with OR=3.4 times, CI=1.1–10.9 times, *p*=0.03). However, there was protective level of government sewage system against *V. cholerae* infections (Table 5).

DISCUSSION

This study provides data on prevalence and potential risk factors of cholera among severe diarrhoea in Sana'a city in Yemen which will be useful to inform decisions for effective control measures. The study results show variability in rates at different age groups, in which there was slightly increasing in the rate of *V. cholerae* infection with increasing age (Table 4). Current study results is similar to that observed in Bangladesh that children cholera more frequently in older children compared to young children during diarrhoeal illness[4]. Higher rate of cholera in older patients might be related to those older children exposed to risk factors that related to out-door activities. When the sources of drinking water versus *V. cholerae* infection were considered, there was a highly significant increasing in the rate of *V. cholerae* infection with Jerry can bottles using (16.1%, with *OR*=3.1 times, *CI*=1.5-6.9, and *p*=0.02) (Table 4). Higher rate of cholera with Jerry can bottles using might be related to faecal contamination of drinking water sources or faecal contamination of the re-used jerry can bottles.

There was a highly significant increasing in the rate of *V. cholerae* infection with dispose sewages to the house surround environment (rate=21%, with *OR*=3.4 times, CI=1.1 – 10.9 times, *p*=0.03) (Table 5).This risk might be related to faecal contamination of drinking water. Bany Al-Harath distract is a densely populated area and has one of the largest concentrations of slums in Sana'a city.

Table 4: The association between *V. cholerae* infections and the sources of drinking water for the patients suffering from severe diarrhoea.

Water sources	*V. cholerae* positive culture (n=28)		OR	CI	χ^2	*Pv*
	No.	%				
Water pump, n=75 (21.7%)	3	4	0.4	0.11-1.3	2.1	0.14
Hand well, n=2 (0.6%)	0	0				
Water grid, n=5(1.4%)	1	20	2.9	0.3-26	0.96	0.32
Stream, n=1(0.3%)	0	0				
Commercial containers, n=131 (38%)	10	7.6	0.8	0.4-2	0.06	0.7
Mineral water, n=11(3.2%)	1	9.1	1.1	0.4-9.2	0.01	0.9
Reused Jerry can bottles, n=81 (23.5%)	13	16.1	3.1	1.5-6.9	8.9	0.02
Crude rate N=345	28	8.1				

OR- odds ratio => 1 (risk), CI- Confidence intervals 1 to more than 1, X^2- Chi-square => 3.9 (significant), *PV* -Probability value =< 0.05 (significant)

Table 5: The association between *V. cholerae* infections and the swages system for the patients suffering from severe diarrhoea.

Swages	*V. cholerae* positive culture (n=28)		OR	CI	χ^2	*Pv*
	No.	%				
Doge hole, n=244 (70.7%)	20	8.2	1.0	0.44-2.4	0.007	0.93
Government sewage, n=73 (21.2%)	4	5.5	0.6	0.2-1.7	0.86	0.35
Dispose to surround environment, n=19 (5.5%)	4	21	3.4	1.1-10.9	4.5	0.03
Crude rate, N=345	28	8.1				

OR- odds ratio => 1 (risk), CI- Confidence intervals 1 to more than 1, X^2- Chi-square => 3.9 (significant), *p* -Probability value =< 0.05 (significant)

Slum settlements often have unhygienic latrines, poor garbage management systems, and sewers that overflow into houses. In most cases, latrines are linked with sewerage lines and municipal water pipes are commonly exposed to sewerage lines which may lead to faecal contamination of the supply water source. In this study, the prevalence of intestinal *E. histolytica* was 50.7%, in which 40.9% of them as single cause and 9.9% were suffering from co-infection with other micro-organisms, current study results were similar compared to previous studies done at Libya and others African countries in which intestinal *E. histolytica* was the most common cause of diarrhea among children[22,23]. High prevalence of intestinal *E. histolytica* is attributed by poor personal hygienic practices and poor environmental sanitation. Also *E. histolytica* and *G. lamblia* can directly transmit through food-handlers to consumers if ingested via contaminated food and water because cysts do not need environmental maturation[24,25]. Finally, for the confirmation of cholera cases, this study used a conventional culture method which remains the gold standard, but this procedure may yield false-negative results in case of inactivation of *V. cholerae* by *in-vivo* vibriolytic action of the phage and/or non-cultivability induced as a result of host response[26,27,28]. Rapid antigen-based diagnostic tests for cholera dipstick assays have identified 0–32% more cases than the conventional culture method in detecting *V. cholerae* antigens in stool samples[26,29,30,31]. By not accounting for culture-negative *V. cholerae* cases we are underestimating total cholera prevalence, but we did not adjust the prevalence calculations for culture negatives because we did not have molecular evidence from this population to estimate the magnitude of the correction.

CONCLUSIONS

V. cholera infection rate, and intestinal protozoa infection rates were significantly high. The findings emphasize that there is cholera epidemic in Sana' city and diarrheal epidemic due to various diagnosed and non diagnosed pathogenic microorganisms which may predispose population of the study to significant health risks. Therefore, constant epidemiological surveillance and applying proper preventive measures through biannual routine parasitological tests and treatment of the infected cases along with the improvement of environmental sanitation are recommended.

ACKNOWLEDGEMENTS

Authors acknowledge the financial support of MHP, Sana'a Yemen, and WHO Sana'a office.

AUTHOR'S CONTRIBUTION

Sheiban AA: writing original draft, conceptualization, methodology, investigation. **Al-Shamahy HA:** Writing, review, and editing, supervision. **Alattab NM:** writing, review, and editing. **Al-Kasem MAA:** writing, review, and editing. Final version of manuscript is approved by all authors.

DATA AVAILABILITY

The datasets generated during this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

None to declare.

REFERENCES

1. Snow J. On the mode of communication of cholera, 1855. Salud publica de Mexico 1991; 33:194–201.PMID: 2053025
2. Sack DA, *et al.* Cholera. Lancet 2004; 363:223–233. *https://doi.org/10.1016/S0140-6736(12)60436-X*
3. Nair GB, *et al.* Spread of *Vibrio cholerae* O139 Bengal in India. J Infect Dis 1994; 169:1029–1034. *https://doi.org/10.1093/infdis/169.5.1029*
4. Paul RC, Faruque ASG, Alam M *et al.* Incidence of severe diarrhoea due to *Vibrio cholevae* in the catchment area of six surveillance hospitals in Bangladesh. Epidemiol Infect 2016; 144(5): 927–939. *https://doi.org/10.1017/S0950268815002174*
5. World Health Organization. Cholera outbreak: assessing the outbreak response and improving preparedness: Global Task Force on Cholera Control; 2004
6. MHP Yemen, Electronic Disease Early Warning System (eDEWS). Weekly Epidemiological Bulletin W46 2017 (Nov 13-Nov 19).*https://doi.org/10.5455/aim.2019.27.85-88*
7. WHO. Cholera surveillance and number of cases. Geneva: WHO; (Accessed 22 August 2017). (http://www.who.int/topics/cholera/surveillance/en/index html).
8. Kimball AM, Wong KY, Taneda K. An evidence base for international health regulations: quantitative measurement of the impacts of epidemic disease on international trade. Revue Scienti fiqueet Technique (International Office of Epizootics) 2005; 24:825–832.
9. MHP Yemen. Relief Web. Yemen–Cholera outbreak - DG ECHO Daily Map | 23/11/2017
10. Lopez AL, *et al.* Cholera vaccines for the developing world. Human Vaccines 2008; 4: 165–169.
11. Clemens JD, *et al.* Field trial of oral cholera vaccines in Bangladesh: results of one year of follow-up. J Infect Dis 1988; 158:60–69. *https://doi.org/10.1093/infdis/158.1.60*
12. Sur D, Lopez LA, Kanungo S, *et al.* Efficacy and safety of a modified killed-whole-cell oral cholera vaccine in India: An interim analysis of a cluster-randomised, double-blind, placebo-controlled trial. Lancet 2009; 374:1694–1702. *https://doi.org/10.1016/S0140-6736(09)61297-6*
13. Andrews JR, Basu S. Transmission dynamics and control of cholera in Haiti: An epidemic model. Lancet 2011; 377:1248–1255. *https://doi.org/10.1016/S0140-6736(11)60273-0*
14. Bertuzzo E, *et al.* Prediction of the spatial evolution and effects of control measures for the unfolding Haiti cholera outbreak. Geophysical Res Let 2011; 38 L06403. *https://doi.org/10.1029/2011GL046823*
15. Tuite AR, *et al.* Cholera epidemic in Haiti, 2010: using a transmission model to explain spatial spread of disease and identify optimal control interventions. Annals Internal Med 2011; 154:593–601.
16. WHO. Cholera vaccines. Weekly Epidemiological Record 2011; 76:117–124.
17. Chaignat CL, Monti V. Use of oral cholera vaccine in complex emergencies: what next? Summary report of an expert meeting and recommendations of WHO. J Health Popul Nutrit 2007; 25:244–261. PMID: 17985828
18. Clemens JD, *et al.* Field trial of oral cholera vaccines in Bangladesh: results from three-year follow-up. Lancet 1990; 335:270–273. *https://doi.org/10.1016/0140-6736(90)90080-O*
19. Sur D, *et al.* Efficacy of a low-cost, inactivated whole-cell oral cholera vaccine: results from 3 years of follow-up of a randomized, controlled trial. PLoS Neglected Trop Dis 2011; 5:e1289. *https://doi.org/10.1371/journal.pntd.0001289*
20. Alam M, *et al.* Seasonal cholera caused by *Vibrio cholerae* sero-groups O1 and O139 in the coastal aquatic environment of Bangladesh. Applied Env Microbiol2006; 72:4096–4104.*https://doi.org/10.1128/AEM.00066-06*
21. WHO. World Health Organization Guidelines for the laboratory diagnosis of cholera. Geneva: WHO Bacterial Disease Unit; 1974. *https://doi.org/10.1371/journal.pntd.0003832*
22. Ali MB, Ghenghesh KS, Ben Aissa R, Abuhelfaia A, Dufani MA. Etiology of childhood diarrhea in Zliten-Libya. Saudi Med J 2005; 26:1759–65.
23. El Ammari NE, Nair GA. Critical evaluation of the intestinal Protozoan parasites among Libyan and other African residents of Al-Khoms, Libya. J Entomol Zool Stud 2015; 3:42–6.
24. Ghenghesh K S, Ghanghish K, Ben Darif ET, Khaled S, Ezzadin F. Prevalence of *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium* spp. in Libya: 2000–2015. Libyan J Med 2016; 11: 10. *https://doi.org/10.3402/ljm.v11.32088*
25. Cheesbrough M. Medical laboratory manual for tropical countries, 2nd ed. Oxford, Butterworth; 1992. *https://doi.org/10.1016/0035-9203(82)90237-1*
26. Alam M, *et al.* Diagnostic limitations to accurate diagnosis of cholera. J Clin Microb 2010; 48:3918–3922. *https://doi.org/10.1128/JCM.00616-10*
27. Colwell RR, *et al.* Viable but non-culturable *Vibrio cholerae* O1 revert to a cultivable state in the human intestine. World J Micro Biotech 1996; 12:28–31. *https://doi.org/10.1007/BF00327795*
28. Faruque SM, *et al.* Transmissibility of cholera: in *vivo*-formed biofilms and their relationship to infectivity and persistence in the environment. Proceedings of the National Academy of Sciences USA. 2006; 103:6350–6355.*http://doi.org/10.1073/pnas.0601277103*
29. Boncy J, *et al.* Performance and utility of a rapid diagnostic test for cholera: notes from Haiti. Diagnostic Microbiol Infect Dis2013; 76:521–523. *https://doi.org/10.1016/j.diagmicrobio.2013.03.010*
30. Ley B, *et al.* Evaluation of a rapid dipstick (Crystal VC) for the diagnosis of cholera in Zanzibar and a comparison with previous studies. PLoS ONE. 2012; 7:e36930. *https://doi.org/10.1371/journal.pone.0036930*
31. Nato F, *et al.* One-step immunochromatographic dipstick tests for rapid detection of *Vibrio cholerae* O1 and O139 in stool samples. Clinical Diag Lab Immunol 2003; 10:476–478. *https://doi.org/10.1128/CDLI.10.3.476-478.2003*