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RESEARCH ARTICLE

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

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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**,** PV=0.02) and reused Jerry can bottles for drinking water (OR=3.1, PV= 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; diarrhoea; prevalence; risk factors, Sana'a city, Saudi Aggression, Yemen.

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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 *Vibrio cholera* and *protozoa causes* among Yemeni patients suffering from severe diarrhea and the potential risk factors of the contracting *Vibrio 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.

Table 1: Age distribution of the patients suffering from severe diarrhoea that tested for *V. cholerae* infection positivity in Sana'a city–Yemen, (July 2017).

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

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). 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 *Entamoeba 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. However, a low prevalence of *EPEC* (0.6%) and *H. nana* (2%) were very low.

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 in Sana'a city–Yemen, (July 2017).

Agents	Frequency	
	Number	Percentage
V.cholerae	28/345	8.1
Single infection	12/345	3.5
Co-infection	16/345	4.6
Entamoeba	175/345	50.7
histolytica	141/345	40.9
Single infection	34/345	9.9
Co-infection		
Giardia	23/345	6.7
lamblia	9/345	2.6
Single infection	14/345	4.1
Co-infection		
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 in Sana'a city–Yemen, (July 2017).

Age groups	*V.cholerae* positive culture (n=28)		OR	CI	χ^2	*Pv*
	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), *PV* -Probability value = < 0.05 (significant)

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 PV=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, PV=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 *PV*=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, *PV*=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. 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.

Table 4: The association between *V.cholerae* infections and the sources of drinking water for the patients suffering from severe diarrhoea in Sana'a city–Yemen, (July 2017).

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 in Sana'a city – Yemen, (July 2017).

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), *PV* -Probability value = < 0.05 (significant)

In this study, the prevalence of intestinal *Entamoeba 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 *Entamoeba histolytica* was the most common cause of diarrhea among children[22,23]. High prevalence of intestinal *Entamoeba 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.

CONCLUSION

Vibrio 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.

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AUTHOR'S CONTRIBUTION

The manuscript was carried out, written, and approved in collaboration with all authors.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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