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RESEARCH ARTICLE

CHOLERA IN SANA'A, YEMEN: CLINICAL FEATURES, RISK FACTORS AND ANTIBIOTIC SENSITIVITY OF *VIBRIO CHOLERAE*

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Abstract

Background: Cholera, caused by *Vibrio cholerae* O1 or O139 serotypes, is one of the most important healthcare associated infections (HAI) among patients in Yemen.
Aim: This study aimed to determine the risk factors associated with cholera outbreaks, clinical presentations, and antibiotic susceptibility of the *V. cholerae* strains isolated among inpatient in Diarrheal Treatment Centers (DTCs) in Sana'a City.
Methods: This is a matched case-control study carried out on 134 DTC inpatients (cases) aged from 2 to 85 years who had a mean age of 26.8 years; and 134 community healthy individuals (control), ranged in age from 2 to 85 years with a mean age of 27.1 years in Sana'a. The identified isolates were tested for antibiotics susceptibility using disc diffusion technique. Data were analyzed using Epi Info 7.2. Express the quantitative data as mean values, standard deviation (SD), when the data are normally distributed.
Results: Among the cases, females are more susceptible to cholera than males (62.7% vs. 37.3%), and there is an increase in the incidence of cholera with age as 32.8% of cases were in the ≥35-year age group. There were significant risk factors for cholera with unwashed fruits (*OR*=33, $p<0.001$), unwashed vegetables (*OR*=5.3, $p=0.001$), outside foods (*OR*=129, $p<0.001$), and leftovers un-cooled food (*OR*=2, $p=0.04$).
Conclusions: Cholera affects all age groups in Sana'a, with females and persons of the age group greater than 35 years mostly affected. The most common clinical presentations were watery diarrhea and abdominal pain. Consumption of unwashed fruits, vegetables, outside food, unrefrigerated food, use of breakdown sewage system, dilapidated sewage near the home. Therefore there is need to ensure that proper hygiene and sanitation to prevent infection.
Keywords: antibiotics susceptibility, Case- control study, cholera, clinical manifestations, DTCs, epidemic, risk factors, Sana'a city, *V. Cholerae*.

INTRODUCTION

Vibrio cholerae is the causative agent of cholera, a Gram-negative, rod-shaped pathogen that can be transmitted between two different environments persisting in saltwater ponds and transmission from the human intestine, transmitted from environmental reservoirs to the human host through contamination of food or water. *V. cholerae* is hypersensitive to low gastric pH, and thus the infective dose of this bacterium is high at more than $x10^8$ organisms[1]. Those cells that escape stomach acid eventually colonize the intestine. The toxin co-regulated pilus (TCP) promotes in colonization by encouraging the formation of bacterial micro-colonies. *V. cholerae* produces cholera toxin (CT), which disturbs the ordinary ion transport of

the intestinal epithelium, resulting in a large influx of water into the intestine resulting in devastating vomiting and diarrhea[2]. The transition between biofilm formation and movement during infection is a key component of the colonization of *V. cholerae*[3]. The main risk factor connected with cholera is restricted approach to clean drinking water and adequate sanitation. As a result, the specific situations in which people live in such suboptimal sanitary conditions were also considered relevant risk factors, such as severe overcrowding, urban slums with limited health infrastructure, and temporary refugee or IDP camps, whether humanitarian or environmental crises and civil unrest[4]. Even with current managements, it is expected that there are more than 3 million cholera cases as well as more than 100,000 deaths annually[5].

In Yemen, there are new published reports indicating the increased prevalence of communicable and non-communicable diseases that are closely related to war, poverty and the collapse of health systems[6-25]. The most recent outbreak of cholera in Yemen began in October 20166[26], and continues until April 2019[27]. In February and March 2017, the outbreak appeared to be declining during the cold weather wave, but the number of cholera cases re-emerged in April 2017[28]. As of October 2018, more than 1.2 million cases have been reported, and more than 2,500 people have died (58% of whom are children in Yemen)[29]. The first cases were mostly in the capital, Sana'a, and some occurred in Aden. By the end of October, cases were reported in the governorates of Aden, Al Hudaydah, Hajah, Ibb, Lahj and Taiz, and in late November also in Al Dhale' and Amran[28,29].

Antimicrobial treatment is optional for critically ill patients and hospitalized patients. It is especially recommended for patients who are severe or moderately dehydrated and continue to pass a large amount of stool during rehydration therapy. Antimicrobial drugs can shorten the period of diarrhea, decrease the volume of rehydration solutions requisite, and shorten the extent of *Vibrio* excretion. Data of the sensitivity of regional strains to antibiotics, if any, be supposed to be used to direct antimicrobial therapy, so antimicrobial susceptibility testing must be performed[30].

The cholera outbreak in Yemen is the largest in the recent history of this disease. The scale of this outbreak can likely be explained by the overall breakdown of public services, including hygiene and sanitation, linked to the war in Yemen. However, evidence of the clinical features, risk factors and antibiotic sensitivity of isolates of *V. cholerae* is needed to guide an appropriate public health response. Therefore this study aimed to identify risk factors for cholera epidemic in inpatients in DTCs, clinical features and antibiotic susceptibility to *V. cholerae* isolated.

SUBJECTS, MATERIAL, AND METHODS

Study design: This a matched case-control study to determine the potential risk factors for cholera infection. Also conducted a laboratory analysis of stool samples to identify the bacterial isolates, and determine the antimicrobial susceptibility pattern of the *V. Cholerae isolates*.

Study area: This study was conducted in cholera treatment centers (DTCs) in Sana'a city. Sana'a is located on a plain of the same name, the Haql Sana'a, which is over 2,300 meters (7,500 ft) above sea level. The plain is roughly 50–60 km long north–south and about 25 km wide, east–west, in the area north of Sana'a, and somewhat narrower further south. To the east and west, the Sana'a plain is bordered by cliffs and mountains, with wadis coming down from them. Sana'a has a population of approximately 3,937,500, making it Yemen's largest city.

Study population: This consist of 134 cholera cases admitted in DTC and 134 community controls identified in Sana'a. The cases and controls were selected and recruited into the study employing the following definitions:

Case: A case was defined as any patient, aged five years and over admitted in DTC from April 2020 to June 2020 with clinical presentations such as vomiting, diarrhea and abdominal pain, and laboratory-confirmed with V. Cholerae infection.

Controls: A control was defined as any person living in Sana'a, age, gender and neighborhood matched with cases without cholera clinical presentations or *V. Cholerae* isolated.

Sample size determination: The estimated sample size of 134 case patients and 134 controls was calculated with the assumption of a projected incidence of potential risk factors of *V. cholera* infection of 25% among case patients and 5% among individual controls from an earlier survey by Sheiban *et al.*,[25] in Yemen, with a confidence level of 99.9% and power of 90% using Epi Info 7.2.

Data collection method: Respondents demographic data (age, sex) clinical presentations (diarrhea, nausea, vomiting, abdominal pain etc.) and risk factors (food, water source, waste disposal system, vaccination status) were collected using a pre-designed structured questionnaire administered by the interviewer.

Laboratory testing: After swab samples were collected from the rectum or stool, the samples were immediately placed in Cary-Blair transfer medium as transport media. All samples were cultured in the Bacteriological Department of the National Center for Public Health Laboratories (NCPHL) Sana'a, using standard bacteriological methods[30,31]. In the laboratory, rectal swabs or stool samples were incubated in alkaline peptone water (APW) at 37 °C for 4 h. Rectal swabs or stool samples were inoculated, and further enriched with broth for 4 h by placing streaks on taurocholate-telluride-gelatin agar (TTGA). Colonies resembling *V. Cholerae* were agglutinated with antigens specific to *V. Cholerae* O1 and *V. Cholerae* O13921[31].

Antimicrobial susceptibility testing: The isolated and identified *V. Cholerae* were tested for susceptibility toamoxicillin (AM) 10 mcg, tetracycline (TE 30 mcg), doxycycline (30 mcg), cefuroxime (CXM 30 mcg), and co-trimoxazole (SXT, 1, 25/23, 75 mcg), ciprofloxacin (CI 5 mcg), nalidixic acid (NA 30 mcg), minocycline (MN 30 mcg), furazolidone (FR 50 mcg),

and erythromycin (ER 15 mcg) (Oxoid Ltd) using the disc diffusion method[32].

Statistical analysis: By using Epi Info statistical program version 6 (CDC, Atlanta, USA) the analysis of data was performed. Expressing the quantitative data as mean values, standard deviation (SD), when the data was normally distributed. Expressing the qualitative data as percentages; Chi square test was used for comparison of two variables to determine the *P* value. Odd ratio (OR) was used with 99% confidence interval to determine risk factors of cholera, *p* value <0.05 was considered statistically significant.

Ethical consideration: From all participants, consents were taken and participants were informed that participation is voluntary and that they can reject this exclusive of stating any reason.

RESULTS

The cases were 37.3% males and 62.7 females. The ages of the cases ranged from 2 to 85 years with a mean age±SD equal to 26.8±16.3 years. Most of the cases were in the age groups 35 years (32.8%) followed by the age group 15-24 years (23.9%) and 5-14 years (22.3%), while only 6 cases (4.5%) were recorded in less than 5 years group (Table 1). Ages in the control group ranged from 2 to 85 years with a mean age±SD equal to 27.1±16.1 years.

Table 1: The distribution of age and sex of cholera patients and control healthy.

	Cholera Cases, n=134		Healthy controls, n=134	
	Number	%	Number	%
Sex				
Males	50	37.3	47	35.1
Females	84	62.7	87	64.9
Age groups (years)				
< 5 years	6	4.5	6	4.5
5-14 years	30	22.3	28	20.9
15-24 years	32	23.9	34	25.3
25-34 years	22	16.5	18	13.5
≥ 35 years	44	32.8	48	35.8
Age variance				
Mean age	26.8 years		27.1 years	
SD	16.3 years		16.1 years	
Median	24 years		24 years	
Mode	35 years		35 years	
Min	2 years		2 years	
Max	85 years		85 years	

Most of the controls were in the ≥35 years (35.8%) age-group followed by the 15-24-year-old (25.3%), 5-14-year (20.9%), and 4.5% under-5-year-old group (Table 1). Females at higher risk of contracting cholera than male (62.7% versus 37.3%), also an increase in cholera incidence with age in which 32.8% of the cases were in age group ≥35 years (Table 1). The most common symptoms among cholera cases were watery diarrhea (98.5%) and abdominal pain (81.3%), followed by nausea (55.2%), vomiting (53.7%) and headache (46.3%), while bloody diarrhea was very low (1.5%), and mucosal diarrhea was 0%; and the mortality rate was zero (Table 2).

Table 2: The frequency and percentage of clinical symptoms and signs among cholera patients.

Symptoms	Number (%)	*p* value
Vomiting	72 (53.7)	NS
Nausea	74 (55.2)	N S
Abdominal pain	109 (81.3)	< 0.001
Headache	62 (46.3)	NS
Watery diarrhea	132 (98.5)	<0.01
Bloody diarrhea	2 (1.5)	NS
Mucosal diarrhea	0 (0.0)	NS
Mortality	0 (0.0)	NS

NS= non-significance > 0.05

There were significant risk factors for developing cholera with unwashed fruits (*OR*=33, *p*<0.001), unwashed vegetables (*OR*=5.3, *p*=0.001), outdoor foods (*OR*=129 *p*<0.001), and un-cooled food residues (*OR*=2, *p*=0.04) (Table 3). When assessing drinking water as a risk factor for cholera; there was no association between all sources of water with cholera infection (Table 3). Collapse of the drinking and sanitation system, dilapidated sanitation near the home, street running for wastewater and contact with diarrhea at home were cholera risk factors (*OR*=4.5, *p* <0.001; *OR*=1.6, *p*=0.046; and *OR*=8.7, *p* <0.001; *OR*=33.3, *p*<0.001; respectively). Positive cholera vaccine was a preventive factor against cholera infection (*OR*=0.11 average protection, *P* <0.001) (Table3). *V. cholera* isolates were 100% resistant to nalidixic acid; amoxicillin; 95.5% erythromycin and 98% for tetracycline, while the rates of resistance were low with cefotaxime (2.2%) and furazolidone (3.8%) (Table 4).

On the other hand, all strains of *V. Cholerae* were 100% sensitive to co-trimoxazole, ciprofloxacin, minocycline and doxycycline (Table 4).

DISCUSSION

In the current study, 134 cases of cholera were confirmed with positive culture diagnosis in a single DTC within 3 months, and this indicates that cholera epidemic is still going on in Yemen, and the fact that the World Health Organization considered the reported numbers of cholera endemic areas to be an

underestimate due to poor Monitoring systems and fear of negative impact on trade and tourism in many countries this is likely to lead to underreporting[33]. Also, this large number of cholera cases that were diagnosed in only one treatment center in the capital, Sana'a, confirmed the considerations of the World Health Organization, which considered these numbers the worst cholera epidemic in modern human history. The mortality rate among the study subjects was zero, although mortality rates are low as a result of intravenous and/or oral rehydration therapy, cholera can cause severe disease due to its rapid onset; populations in low-income areas such as Yemen are particularly at risk of infection in areas where public health systems cannot handle outbreaks, as well as where around 60% of the public health system has been destroyed due to Saudi/UAE aggression against Yemen 6 years ago which is still continuing[33,34].

Table 3: The associated risk factors of contracting *V. Cholera* among patients attending DTCs and healthy controls in Sana'a city.

Risk factors	Cases n=134		Controls, n=134		*OR*	*95% CI*	X^2	*p*
	No	%	No	%				
Food consumed								
Unwashed fruits	45	33.6	2	1.5	33	7.8-141	47	<0.001
Unwashed vegetables	19	14.2	4	2.98	5.3	1.7-16.2	10.7	0.001
Outdoor food	66	49.2	1	0.75	129	17.5-950	84	<0.001
un-cooled food residues	4	2.98	0	0	2	1.7-2.2	4	0.04
Bottle water	2	1.5	1	0.75	2	0.1-22	0.33	0.56
Mineral water	43	32.1	65	48.5	0.5	0.03-0.8	7.5	0.006
Tap water	89	66.4	112	83.6	0.33	0.2-0.6	10.5	0.01
Travel in the past 5 days	5	3.7	5	3.7	1	0.2-3.5	0.0	1.0
House water source								
Water pump	81	60.44	93	69.4	0.6	0.4-1.1	2.3	0.12
Hand well	1	0.74	0	0	2	1.7-2.2	1.0	0.31
Water grid	0	0	0	0	undefined		1.0	0.31
Stream	0	0	0	0	undefined		1.0	0.31
Commercial containers	0	0	13	9.7	0	0 -0.2	13	<0.001
Mineral water	17	12.7	11	8.2	1.6	0.7-3.6	1.4	0.2
Sabial water	35	26.1	10	7.5	4.3	2-9.2	16.7	<0.001
Sewage disposal								
Pipes	25	18.7	41	30.6	0.5	0.2-0.9	5.1	0.02
Sewers	109	81.3	92	68.6	1.9	1.1-3.5	5.7	0.01
Breakdown of potable and sanitation system	27	20.1	7	5.2	4.5	1.9-10.9	13.4	<0.001
Dilapidate sanitation near the house	63	47	47	35.1	1.6	1-2.6	3.9	0.046
Swage run in the street	77	57.5	18	13.4	8.7	4.7-15.9	56.7	<0.001
Having diarrhea contact at home	45	33.6	2	1.5	33.3	7.8-141	47.7	<0.001
Cholera vaccine positive	5	3.7	34	25.4	0.11	0.04-0.3	25.2	<0.001

Study results showed variation in rates across different age groups, as there was a slight increase in the incidence of *V. Cholerae* with age (Table 1). The results of the current study are similar to those previously observed in Yemen[25], while in Bangladesh and other parts of the world; cholera was more common among younger children than in older children and adults[35].

Table 4: Antibiotic sensitivity test for isolated *V. cholera* from cholera patients.

Antibiotics	Sensitive	Resistant
	No (%)	No (%)
Co-trimoxazole (SXT, 1, 25/23, 75µg),	134 (100)	0 (0.0)
Ciprofloxacin (CI 5µg)	134 (100)	0 (0.0)
Minocycline (MN 30 µg)	134 (100)	0 (0.0)
Nalidixic acid (NA 30 µg)	0 (0.0)	134 (100)
Tetracycline (TE 30 µg),	132 (98.5)	2 (1.5)
Doxycycline (DO 30 µg),	134 (100)	0 (0.0)
Amoxacillin (AM) 10 µg	0 (0.0)	134 (100)
Cefotaxime (CTX 30 µg)	131 (97.8)	4 (2.2)
Furazolidone (FR 50 µg)	129 (96.2)	5 (3.8)
Erythromycin (E 15µg)	6 (4.4%)	128 (95.5)

The higher incidence of cholera in older patients may be associated with exposure to risk factors associated with outdoor activities. Obtained result also contradicts the finding of Sack *et al*., children were more likely to be infected, and children aged two to four years had the highest rates of cholera[36]. Females were more likely to have cholera in current study as females had 62.7% of cholera cases versus 37.3% for males (Table 1). These findings differ from those reported in cholera pandemics in the past 20 years, in Africa, Iraq, India and Vietnam[37-39] where the incidence of cholera is approximately equal in both sexes.

In the current study, abdominal pain and watery diarrhea occurred significantly (81.2% and 98.5%; $p < 0.01$, respectively) (Table 2). However, other typical cholera symptoms like vomiting etc. were less frequent. In severe cases of cholera, cholera patients may experience lethargy and may have sunken eyes, dry mouth, cold or wet skin, or hands and feet. Kussmaul's breathing, a deep and exhausting breathing pattern can also be caused by acidosis from faecal bicarbonate loss and lactic acidosis associated with poor perfusion. Blood pressure declines as a result of dehydration, peripheral pulse is fast and strenuous, and

urine production reduces eventually. Muscle cramps and weakness, altered consciousness, seizures, or even coma due to an electrolyte imbalance are frequent, particularly in particular in children[36]. But all of our cases did not develop these symptoms due to prompt, appropriate DTC treatment.

The approximation of cholera prevalence is on the whole important to take effective control measures, comprising the provision of clean water, enhanced hygiene and sanitation, and presentation of cholera vaccines. Oral cholera vaccines have been found to be safe and effective[40-42]. In current study there was protection role for vaccination in which the vaccinated individual had *OR* equal to 0.1, $p<0.01$(Table 3). Nevertheless, representation studies have revealed that sanitation measures and water may afford an equally viable solution, particularly in the long term, because the immunization approved by vaccines declines over time[43-45]. Two inactivated types of cholera vaccines are presently offered: one have killed cholera whole cells (rBS-WC) and recombinant cholera toxin B subunit; and the other have only killed cholera whole cells (WC)[46,47]. Field trials confirmed that the two vaccines afforded >50% protection for 3 yrs[47,48]. In spite of this, the WC vaccine is cheaper, (US$1.85 per dose) in the public division, with a protective effectiveness of 66% all through the third year of follow-up, as described in a study from Kolkata, India[49]. Realistic data concerning incidence of cholera is at present unavailable in Yemen, which limits the validity of any cost effectiveness evaluation of a potential intervention programme.

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 sabil water (*OR*=4.1) (Table 3). Higher rate of cholera with this type of water might be related to faecal contamination of this source. There was a highly significant increasing in the rate of *V. Cholerae* infection with breakdown of potable and sanitation system (*OR*=4.5), dilapidate sanitation near the house (*OR*=1.6) and swage run in the street (*OR*=8.7) (table 3). These risks might be related to faecal contamination of drinking water from sanitation system. Madbah area (the center study) 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.

The main objective of this study was to analyze trends in multiple antibiotic resistances among clinical strains of *V. cholerae* isolated in 2019-2020 in Sana'a city, Yemen. Even though the therapy for cholera is principally supportive, antimicrobial therapy can be useful in decreasing the volume of stools and length of illness[50,51]. While tetracycline has been the mainstay of therapy, erythromycin, furazolidone and co-trimox-azole are the other reported alternatives[52,53]. Multidrug resistant classical *V. cholerae* strains and simultaneous epidemic outbreaks of classical of *V. cholerae* has been reported in Yemen. Majority of the classical 01-0139 strains in this study was resistant to ampicillin and erythromycin and a similar trend of resistance was seen in Bangladesh and India[54]. All strains of *V. cholerae* in current study were sensitive to co-trimoxazole; since cholera is a non-invasive disease, drugs such as co-trimoxazole, which is not absorbed from the gastrointestinal tract, must be good choice for treatment of cholera in Yemen. Most strains isolated in current study were sensitive to tetracycline (98.5%) (Table 4). This result is on contrast with that of India, Latin America, Tanzania, Bangladesh and Zaire; in which high incidence of *V. cholerae* non-O1, non-O139 strains resistant to tetracycline were reported[25,55-58]. In the current study ciprofloxacin was 100% effective against isolated strains of V. *Cholerae*. Reservation about promotion of ciprofloxacin as a first line drug for the treatment of cholera in developing countries has been expressed[59], since it is an important substitute drug for treatment of multidrug resistant enteric and other pathogens. Extensive use of this drug and empirical therapy for treating diarrheal infection might have promoted incidence of ciprofloxacin resistant *V. cholerae*, which has emerged for the first time in India during 1992 among *V. cholerae* non-O1 non-O139 and during 1995 among *V. cholerae* O1 and O139 strains[60].

Sundaram & Murthy[61] reported that only 2±7% non-O1 isolates were multi drug resistant in Madras; an area endemic for cholera in south India, but none of the strains was resistant to nalidixic acid. In the current study, it was observed that all -O139 isolates exhibited resistance to nalidixic acid. It is amply clear that long-term surveillance programmers are essential to identify changes in the spectrum of microbial pathogens causing serious infection and to monitor trends in antimicrobial resistance patterns[62-64].

Limitations

This research suffers from specific limitations ranging from common flaws (not all results and answers to all research questions were justified) due to the lack of previous studies to compare with it in Yemen even in cholera-endemic areas in recent times. There were methodological problems as the study time was short (3 months), and the molecular methods for typing *V. cholerae* isolates and for antibiotic sensitivity testing was not performed, so further studies in a larger sample size with the use of the molecular technique is recommended in further studies.

CONCLUSIONS

Cholera affects all age groups in Sana'a, with females and persons of the age group greater than 35 years mostly affected. The most common clinical presentations were watery diarrhea and abdominal pain. Consumption of unwashed fruits, vegetables, outside food, unrefrigerated food, use of breakdown sewage system, dilapidated sewage near the home, running of street sewage, and contact with diarrhea in the home were risk factors, while cholera vaccination was protective against it. So, there is the need to ensure that proper hygiene and sanitation to prevent infection.

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AUTHOR'S CONTRIBUTIONS

Al-Mohanadi EMA: writing original draft, literature survey, filed work in hospitals. **Moharem ASS:** methodology, conceptualization. **Al-Moyed KAA:** formal analysis, review. **Benedict CC:** investigation, data interpretation. **Al-Shamahy HA:** critical review, supervision. **Al-Haidari SA:** data curation, investigation. **Al-Hadad AA:** data curation, investigation. All authors revised the article and approved the final version.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

None to declare.

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