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Epidemiological study of the cholera outbreak in the city of Erbil- Kurdistan region / Iraq

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Abstract

Background and objective: Cholera is a highly contagious acute diarrheal infection caused by Gram negative *Vibro cholerae*. A number of disease outbreaks are recorded annually in different countries around the world. The aim of current study is characterization of the 2022 cholera outbreak in the city of Erbil, Kurdistan region/ Iraq.
Methods: The data of2,192 suspected cholera patients who were admitted to the hospital in the period from June 2022 to September 2022.
Results: The results show that 205 (9.4%) of the hospital-admitted patients were identified as cholera positive, and they have complete data. The mean age ±SD of cholera cases was of 35.71 ± 21.9 years old. No association between males and females regarding the age distribution of cholera cases, and the percentage of male-to female-patients were close together (50.2% males to 49.8% females). Moreover, the etiology of the outbreak was identified as *V. cholerae* O1 serotype Ogawa. The antibiotic sensitivity test of the isolates showed that the strains are sensitive towards meropenem (10µg), Gentamicin (10µg), and Ciprofloxacin (10µg), whereas all the tested bacterial samples shown to be resistant to trimethoprim.
Conclusion: The etiology of the cholera outbreak in the city of Erbil was *V. cholerae* O1 serotype Ogawa. Around half of the infected people were aged between 20 to 44 years old. This is the first report on the recent outbreak in the Erbil-Iraq.
Keywords*:* Cholera; Vibriocholerae; Erbil; Bacterial infections.

Introduction

Cholera is a highly contagious disease caused by gram negative bacterium called *V. cholerae*.(1) The bacterium is known to be able to thrive in aquatic environments and can cause severe form of diarrhea which might potentially lead to death.(2)
The clinical manifestation of this condition characterized by watery diarrhea include the expulsion of significant amounts of liquid stool, imbalances in electrolyte levels, swift onset of dehydration that may progress to hypovolemic shock, and metabolic acidosis.(3)
Despite the existence of over 200 serogroups of *V. cholerae*, cholera outbreaks are predominantly attributed to two serogroups, namely O1 and O139. The O1 serogroup can be further categorized into classical and El Tor biotypes, each with Ogawa and Inaba serotypes.(4) In spite of the genetic homogeneity of El Tor vibrios, recent studies utilizing whole-genome sequence (WGS) analysis have revealed the existence of multiple lineages or transmission types. Additionally, the emergence of novel El Tor hybrids, as well as variations in the B subunit of the CT encoding gene (ctxB) and polymyxin-B sensitivity, has further complicated the

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differentiation of El Tor biotypes. Notably, outbreaks of cholera in Asia and Africa have been increasingly linked to atypical El Tor variants exhibiting genetic variability in CTX phages, rstR genotypes, and single-nucleotide polymorphisms (SNPs) throughout the genome of *V. cholerae*.[5,6]

The impact of cholera on developing countries population is still high, with 323,369 cases, and 857 deaths were reported from 24 countries only in 2020.[3,7] Cholera continues to pose a significant risk to numerous nations worldwide, particularly in Asia, Africa, and Hispaniola.[8,9]

Cholera outbreaks and epidemics can often related with natural disasters, conflicts, and refugee camps, where inadequate sanitation, lack of safe drinking water and hygiene conditions prevail.[10,11] Several developing countries, including Iraq, have faced periodic outbreaks of cholera recently.[12] The destabilizing effects of war, malnutrition among the general population, inadequate sanitation and hygiene infrastructure, and the mass displacement of people in Iraq, compounded by the impact of global warming, have collectively led to a surge in cholera cases.[13,14] From 2000 to 2022, Iraq experienced five cholera peaks occurring in 2003, 2007, 2012, 2015, and 2022.[15,16]

The last outbreak was in the year 2022, in which Iraq had experienced a fresh wave of cholera outbreaks in various cities.In June 19th, 2022, Iraqi health authorities have reported a concerning surge in cholera cases with 13 confirmed cholera infection; ten of the cases were recorded in the city of Sulaymaniyah, two in the city of Muthana, and one in the city of Kirkuk. As of 24 July 2022, the number of the cases were escalated and the World Health Organization (WHO) reported a total of 449 positive cholera cases with three associated deaths.[17] By August 7th, 2022, the number of cholera cases had skyrocketed to 783, resulting in four fatalities. Kirkuk accounted for a significant portion of the cases, with 450 infections and three deaths reported, while the city of Baghdad-Rasafa recorded 193 cases and one death. In contrast, Thi-Qar saw a comparatively lower number of cases, with only 52 reported instances of cholera.[12,13,15-17]

The objective of the present study is to investigate the epidemiological characteristics of the cholera outbreak that occurred in the Erbil governorate, Iraq in 2022.

Methods

Sample Collection

Based on the Iraqi Standard Operating Procedures for Laboratory Identification of *V. Cholera* (USAID, SOP: NCL - BE 00110)[18], stool specimens were collected from suspected patients in the city of Erbil during the period of June to September 2022. Samples were stored in the alkaline peptone water (APW, pH= 8.6) for 4 to 6 hours at 37 °C.

Diagnosis of the pathogen

Preliminarily bacterial identification was carried out by sub-culturing a sample from APW onto the *V. cholerae* selective media which is thiosulfate citrate bile salts sucrose (TCBS) agar and incubated the culture at 37°C for 18–24 hours. The suspected *V. cholera* colonies showed as flat, yellow, translucent colonies with raised centers.[19] The colonies were separated and used to do the following biochemical tests such as: the oxidase test,[20] and the Kligler's Iron Agar (KIA) test.[21]

Serological test

Freshly grown colonies on APW were tested to identify the serology using polyvalent antisera against the *V. cholera* serogroups O1 and O139. *V. cholerae* O1 was recognized as agglutinated in polyvalent antiserum to the O1 serogroup. *V. cholerae* O1 was subsequently tested for agglutination in the monovalent Ogawa and Inaba antisera in order to determine the serotype of the serogroup.

Antibiotic susceptibility test

The antibiotic susceptibility test was carried out for four of the randomly chosen

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samples using Kirby-Bauer disk diffusion susceptibility test following the previously described protocol.[22] Briefly, 0.5 MaFarland of *V. cholera* samples were spread on Mueller-Hinton (MH) agar.

The antibiotic discs that were used in this study were: tetracycline (10 µg), Vancomycin (30 µg), Meropenem (10 µg), Gentamicin (10 µg), Ciprofloxacin (10 µg), Doxycycline (10 µg), Rifampicin (5 µg), Trimethoprim (10 µg). The disc was placed on the plates on the MH agar and incubated at 35°C for 18 hours. The bacterial inhibition zones on the plates were measured using the rulers to the nearest millimeter.[22]

Statistical analyses

XLSTAT software version 2019.2.2 has been used to analyze the data. Descriptive statistic was adapted for presenting the mean and standard deviation. The frequency and percentage were presented by a bar chart, and pie chart.

The statistical inference was dependent on the Chi-square test for testing the significant differences between variables. Differences were considered statistically significant when the *P*-value ≤0.05.

Results

Epidemiological data

From June to September of 2022, there were 2,192 suspected cholera cases admitted to the Erbil Teaching Hospital in Erbil City, Iraq. Among them, only 205 cases were recorded with complete data. Results show, among those 205 positive cholera cases; the number of diagnosed cases in June, July, August, and September was 34,49,89 and 33 respectively (Figure 1 A). Additionally, the total gender distribution of the diagnosed patients was 103 (50.2%) males to 102 (49.8%) females (Figure 1 B).

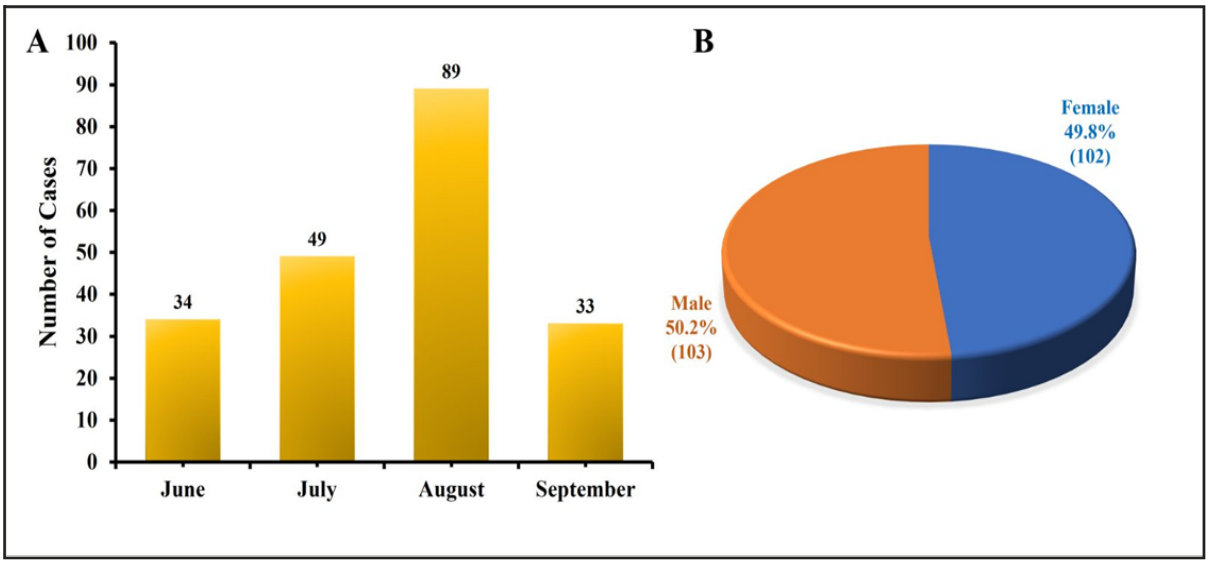

Figure 1 (A) Bar chart of the monthly diagnosed cholera cases in Erbil. (B) The pie chart represents gender distribution of the diagnosed cholera case.

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Results show that the mean age ±SD of cholera cases was 35.71 ± 21.9 years old. Table 1 shows, that 46.8% of affected people were aged between 21,and 50 years, and 20 cholera cases in males were present in the age group 31 to 40, while in females 20, and 20 cases were recorded in the age group 11 to 20 and 41 to 50 respectively, and there were no significant differences between age categories regarding the gender distribution (*P* value =0.171).

Bacteriological and serological identification

The positive cholera cases were confirmed using both bacteriological and serological methods. Preliminary identification was carried out by visualizing the yellow shiny colonies of the V. cholerae on the TCBS agar (Figure 3A). The positive cases were confirmed when the colonies of the tested samples showed the oxidase-positive, acid/alkaline, no gas, and no H2S (Figure 3 B).

The serogroup and serotype of the pathogen were confirmed by slide agglutination test with polyvalent O1 or O139 antisera. The results showed a positive agglutination for the polyvalent O1 antiserum and monovalent Ogawa was a serotype.

Table 1 Number and percentage of people of different ages that faced cholera disease for both genders

Age (Year)	Male		Female		Total Number (%)	*P*-Value
	Number	%	Number	%		
10 ≥	18	66.7	9	33.3	27(13.2)	
11 to 20	15	42.9	20	57.1	35(17.1)	
21 to 30	14	48.3	15	51.7	29(14.2)	
31 to 40	20	66.7	10	33.3	30(14.6)	0.171
41 to 50	17	45.9	20	54.1	37(18.0)	
51 to 60	7	41.2	10	58.8	17(8.3)	
+61	12	40.0	18	60.0	30(14.6)	
Total	103	100	102		205	

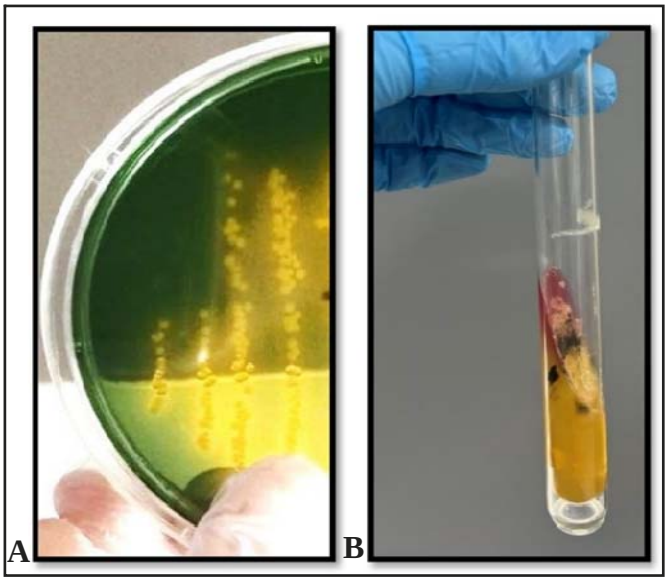

Figure 3 An example of *V. cholerae* plate grown on (A) TCBS agar and (B) KIA

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Antibiotic susceptibility test (AST):
The results of the AST showed that all the tested bacterial samples (100%) were sensitive to meropenem (10 µg), gentamicin (10 µg), and ciprofloxacin (10 µg), however, all the samples were resistant to trimethoprim (10 µg). Only 25% of the samples were shown to be resistant to tetracycline (10 µg); 50% were resistant to vancomycin (30 µg); and 75% of the samples were resistant to doxycycline (10 µg) and rifampicin (5 µg) (Figure 4).

Discussion

Cholera is a significant worldwide health issue, causing around 100,000 deaths and impacting roughly three million individuals each year;[7] This disease remains a significant public health concern in numerous regions across the globe including Iraq where multiple outbreaks have occurred during the past two decades.[12,15,16] The current study was designed to investigate the epidemiological and bacteriological aspects of the most recent cholera outbreak in the Erbil governorate, in Iraq's Kurdistan region.
Based on the findings of the current investigation, only a small proportion (n 205, 9.4%) of the 2,192 cases were determined to be positive for cholera. Comparable studies conducted in different countries have also reported relatively low percentages of cholera cases among diarrhea cases. For instance, in a study carried out to investigate the cholera outbreak in the city of Sulaiymahyah, Iraq in 2022 shows that only 8.7% of the total 4,754 suspected cases were positive for cholera.[16] Additionally, a study carried out at a Kathmandu University teaching hospital in Nepal, 31% (46 out of 148) of stool samples from suspected patients with acute diarrhea were positive for *V. Cholera* serogroup O1, biotype El Tor, serotype Ogawa.[23] Additionally, other pathogens such as *Entamoebahistolytica*, *Giardia lamblia* can cause acute diarrhea in human being and *E. histolytica* has already been described among the children suffering from diarrhea in the cities of Duhok and

Figure 4 An example of Kirby-Bauer disk diffusion susceptibility test carried out for a clinically isolated *V. cholerae*. The antibiotic disks abbreviations are as follow: TE: Tetracycline (10 µg), VA: Vancomycin (30 µg), MEM: Meropenem (10 µg), CN: Gentamicin (10 µg), CIP: Ciprofloxacin (10 µg), DO: Doxycycline (10 µg), Ra: Rifampicin (5 µg), TMP: Trimethoprim (10 µg)

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Kirkuk.[24] Based on the WHO 2022 report, the major source of contamination for the last 2022 cholera outbreak in Iraq were contaminated water sources such as water from tankers sold to people, house tanks, artesian wells, and shallow wells in mosques.[25]

During the period of four months of the study, the highest positive cases were recorded in August (43%), followed by July (24%), whereas the least positive cases were recorded in September (16%). All the isolates from this study were identified serologically as belonging to the same biotype O1 serotype Ogawa. Studies conducted in the seven instances of cholera epidemics that took place in the Kurdistan region from 1995 to 2022, and results demonstrated that all of these outbreaks were attributed to the presence of *Vibrio cholerae* serotypes Inaba and Ogawa of the O1, El Tor strain.[26-28]

A comprehensive investigation of the cholera epidemic that occurred in the Sulaymaniyah province of Iraq between June and July 2022 identified the causative strain as *V. cholerae* Ogawa serotype.[16] A study showed that *V. cholera* O1, biotype El Tor, and serotype Inaba were the cause of the cholera distribution in Baghdad in 2015.[26] In a separate investigation, 80 clinical *V. cholerae* isolates and five environmental isolates obtained during the Iraqi outbreak from 2007 to 2009 were analyzed. Notably, all the environmental isolates were identified as non-O1 serotype, while 55% of the clinical isolates exhibited the same serotype.

It is known that temperature and climate changes can affect the spread of different infectious diseases including cholera, and high temperature and low precipitation provide an optimal condition for the *V. cholearae* proliferation and spread.[29] The lower recorded of positive cholera cases in September can be related to the decreasing of the temperature in the city of Erbil and/or the governmental action plans to control the spread of the disease through raising public awareness, prohibiting the use of leafy vegetables in the fast-foods and restaurants, and promoting the use of bottled water instead of tap water for drinking.

The gender distribution results of this study show that the number of the positive cases among males was slightly higher than among female (male 50.2%, female 49.8%). Although cholera can equally affect both men and women; different reports from different countries have shown different patterns of the disease among the genders. For example, the majority (60%) of the women were shown to be positive for cholera in the Bauchi State, in the north part of Nigeria.[30] Whereas, the higher percentage of the cholera cases were recorded among men in Sierra Leone's population[31] and in Sulaymaniyah province of Iraq[16]. As it was discussed by Rancourt N. (2013),[17] the gender distribution difference of the positive cholera cases can be related to several factors including the life styles, eating habits, and Socio-demographic location of the studies.

The present study shows, 46.8% of cholera cases were recorded in ages 21 to 50. According to the study of Sabir et al., in Sulaymaniyah province during June and July 2022, the highest proportion (over 60%) of cholera cases were recorded among individuals aged 20 to 44 years,[16] however; the majority of the cholera positive cases were among the children younger than 10 years old during cholera outbreak in Uganda (2011-2015).[32] Similar to the gender difference, the difference in positive cases among different age groups can be related to the life style and eating habits of different age groups, particularly people around the age of 20 to 44 who generally tend to eat outside, which may increase their risk to get infected with cholera. No mortality was recorded in related to this cholera during the data collection time in Erbil. Although four cholera-related death were recorded in whole Iraq by August 14, 2022.[17]

The results of the antibiotic sensitivity test

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revealed that all the tested isolates exhibited sensitivity towards meropenem, gentamicin, and ciprofloxacin. Conversely, resistance to trimethoprim was observed in all the samples. Tetracycline exhibited resistance in only 25% of the samples, while vancomycin showed resistance in 50% of the tested samples. Furthermore, 75% of the samples displayed resistance to doxycycline and rifampicin. An investigation into the cholera outbreak that occurred in the Thi-Qar Province of Iraq between 2015 and 2016 reported that the isolated strains were resistant to ampicillin (100%), followed bynalidixic acid (90%) and sulfamethoxazole-trimethoprim (80%), while they demonstrated moderate levels of resistance to tetracycline and ciprofloxacin (55%) and chloramphenicol (45%).[33]

A study showed a significant increase in the Ciprofloxacin resistance among *V. cholera*, with minimum inhibitory concentration (MIC) values exceeding 4 µg/ml. However, the majority of the isolates remained susceptible to other commonly used antibiotics, such as gentamicin, tetracycline, and chloramphenicol.[34] Another study examined changes in antimicrobial susceptibility trends of *V. cholerae* strains isolated between 2006 to 2016 in Nepal and observed that ampicillin resistance declined from 93% (2006) to 18% (2010), then increased to 100% (2016); cotrimoxazole resistance remained constant ranging from 77%-100%; nalidixic acid resistance was 100% since 2006; ciprofloxacin and tetracycline resistance peaked during 2010-2012 and declined to 0% by 2016.[35] The results of a meta-analysis indicated that the prevalence of *V. Cholera* resistance to meropenem was found to be only 3%.[36] Genome sequence analyses have revealed an increasing prevalence of acquired trimethoprim resistance genes that encode for trimethoprim-insensitive homologs of the sensitive dihydrofolate reductase.[37]

An analysis conducted to assess resistance rates in environmental samples of *V. cholerae* O1/O139 isolates, has revealed that 14% of these isolates were resistant to tetracycline.[38] The rampant utilization of antibiotics has led to the emergence of various resistance mechanisms in gram-negative bacteria, including *V. cholerae*.[39]

Conclusion

This study is the first report on the latest outbreak of V. cholerae in the Erbil governorate, Iraq, which occurred in 2022. Epidemiological analysis revealed a significant increase in V. cholerae cases in Erbil in August and July 2022, compared to June and September of the same year Regarding age distribution, 46.8% of affected cholera were aged between 21 to 50 years, with a slightly higher percentage of male patients than female patients. The causative strain of the outbreak was identified as V. cholerae O1 serotype Ogawa. Antibiotic sensitivity testing showed that the strains were susceptible to meropenem, gentamicin, and ciprofloxacin, but resistant to trimethoprim. These findings highlight the importance of antibiotic sensitivity testing in guiding appropriate treatment for patients during future outbreaks and the need for relevant authorities to develop preventive measures based on the identification of causative strains to mitigate similar outbreaks in the future.

Competing interests

The authors declare that they have no competing interests.

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