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Identification of Vibrio Cholerae Serotypes and Epidemiological Trends During Cholera Outbreaks in Kerbala Province, Iraq

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Abstract

Cholera is an acute diarrheal disease caused by Vibrio cholerae, a gram-negative, comma-shaped bacterium. It causes severe, large-volume watery diarrhea, leading to dehydration and death if left untreated. It spreads through contaminated food and water via the oral-fecal route.

Aims: The present study aimed to compare cholera outbreaks in Kerbala city during 2015 and 2017, focusing on their duration, bacterial serotypes, and the role of local treatment plants as water sources in transmission.

Methods: This descriptive study was conducted among patients affected during outbreaks in September-November 2015 and during November 2017. It included 7609 patients in 2015 and 920 in 2017, along with 250 and 80 water samples collected from different sources during the respective cholera outbreak periods. Bacterial strains were isolated from stool and water samples and confirmed by culture and serotyping. Data collection involved direct patient visits and retrospective review of medical records. Inclusion criteria were based on physicians' clinical suspicion and adherence to the case definitions and detection protocols of the National Cholera Control Plan and the World Health Organization.

Results: Two cholera outbreaks occurred in Kerbala. The bacterial strains were isolated, serotyped, and confirmed from stool and water samples. Water from water purification plants (tap water) and rivers was tested. A total of 188

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laboratory-confirmed cholera cases were recorded during the 2015 outbreaks, and 21 of 250 water samples from rivers tested positive. In contrast, during the 2017 outbreak, 76 confirmed cholera cases were reported, with no positive findings among the 80 water samples collected from all water sources. A relatively short period of cholera incidence was observed, spanning from autumn through early winter (September to the end of November). During the 2015 outbreaks, cholera incidence was observed during a relatively short seasonal extension from early autumn to late November. Although 3 serotypes (Inaba and Ogawa) were reported in patients, NAG was reported in water sources. During the 2015 outbreaks, water samples were positive; during the 2017 outbreaks, water samples from different sources were tested for cholera, with none testing positive. Ultimately, all strains recovered during the 2015 and 2017 outbreaks were found to belong to a single serotype (Inaba).
Conclusion: Cholera cases in Kerbala decreased from 2015 to 2017, underscoring the impact of public health interventions. Nevertheless, remaining essentially vigilant community health and reducing healthcare burdens, but ongoing vigilance remains vital during mass gatherings and seasonal peaks.

Keywords: Epidemiology, outbreak, serotypes, Vibrio cholerae

INTRODUCTION

Cholera remains a significant public health challenge in many countries (1, 2). In September 2015, an outbreak was officially declared in Iraq, with 4,945 confirmed cases across 17 of the 18 governorates (3, 4). Cholera is an acute intestinal infection caused by the bacterium Vibrio cholerae and affects both adults and children, often resulting in profuse watery diarrhea (5, 6).

Transmission occurs primarily through ingestion of food or water contaminated with the pathogenic bacterium Vibrio cholerae. Annually, there are an estimated 3 to 5 million cholera cases globally, leading to approximately 105,000 to 120,000 deaths (7). The incubation period ranges from two hours to five days, which explains the rapid onset and explosive cholera epidemics (8).

Approximately 75% of infected individuals remain asymptomatic; however, they may continue shedding the bacteria in their feces for up to 2 weeks, contributing to sustained transmission. Cholera can cause severe dehydration and death within hours if untreated, especially in children and adults (9).

Since the start of the millennium, the number of cholera cases worldwide has consistently increased, with outbreaks persisting in Sub-Saharan Africa. The Eastern Mediterranean region, particularly several conflict-affected countries, continues to face major challenges due to limited access to safe drinking water and poor sanitation. Iraq's vulnerability to cholera epidemics is heightened by its geographic position along major pilgrimage routes to Mecca and the presence of numerous religious shrines that attract large mass gatherings. The first documented cholera outbreak occurred in Iraq in Basrah in 1820, which was associated with high mortality rates (10, 11).

Cholera reemerged in August 1966 after a prolonged absence, marking the onset of the seventh global pandemic of cholera (12, 13).

Rapid response measures implemented during the 2015 outbreak significantly curtailed the epidemic and limited its spread (14). According to Mr. Altaf Musani, the acting WHO Representative for Iraq, WHO remains committed to collaborating with the Iraqi Ministry of Health and partners to implement effective cholera prevention and control interventions (15). Looking ahead, it's important to review and evaluate previous response efforts and ensure adequate preparations, such as ensuring key essential medicines, medical supplies, and diagnostic laboratory capacity are in place to address future outbreaks effectively (3).

METHODS

Patients and Study Design: A descriptive study was conducted among patients affected during cholera outbreaks in Kerbala city in 2015 (from 18 September to 11 November) and in 2017 (from 12 to 30 November). The study included 7609 patients during the 2015 outbreaks and 920 patients during the 2017 outbreak. It also included 250 and 80 water samples collected from various sources during the 2015 and 2017

outbreaks, respectively. Bacterial strains were isolated from stool and water samples collected during the outbreaks and confirmed by culture and serotyping. Data collection encompassed direct patient visits to health care facilities and retrospective reviews of medical records during both outbreak periods. Inclusion criteria were based on physicians' clinical suspicion and strict adherence to the case definitions and detection protocols established by the National Cholera Control Plan (NCCP) and the World Health Organization.

Case definition

Every year, the Iraqi Ministry of Health issues the National Cholera Control Plan (NCCP) and distributes it to all governorates (10). This strategy adopts the World Health Organization's definition of a cholera outbreak. According to the WHO, "a cholera outbreak should be suspected when there is a sudden increase in the daily number of patients presenting with acute watery diarrhea, especially those producing the characteristic 'rice-water stool' associated with cholera". Confirmation of an outbreak is supported by the isolation of Vibrio cholerae O1 or O139 from the stool of any patient presenting with diarrhea.

Case detection

The NCCP provides standardized guidelines for specimen handling and laboratory diagnosis. Stool samples collected at primary health centers (PHCCs) are transported in Cary–Blair transport medium to the nearest hospital laboratory. In the hospital laboratory, stool samples from both PHCCs and hospital admissions are processed on TCBS (thiosulfate–citrate–bile salts–sucrose) agar to identify V. cholera. Stool samples that test positive are subsequently forwarded to the Central Public Health Laboratory reference facility for confirmatory testing and further characterization.

Every confirmed case of cholera is reported to the national Communicable Disease Control Center in Baghdad. The NCCP improves coordination and collaboration among all relevant sectors and stakeholders during an epidemic situation and strengthens surveillance activities. These activities include issuing a weekly report on diarrheal diseases; ensuring that all suspected cholera cases in hospitals and PHCCs (routine situation) are tested in accordance with the case definition; and maintaining daily routine monitoring of drinking water chlorine levels by PHCCs.

Outbreak in 2015 & 2017

The National International Health Regulations (IHR) Focal Point of Iraq notified the WHO of additional laboratory-confirmed cholera cases. As of November 2015, a total of 2,810 laboratory-confirmed cases of Vibrio cholerae O1 Inaba had been reported to the Central Public Health Laboratory in Baghdad, originating from 17 governorates nationwide.

In Kerbala city, 188 confirmed cases were identified and grouped into 3 serotypes: (Inaba, Ogawa, and NAG). These isolates were detected in stool samples, river water, and treated tap water. V. cholerae NAG may cause a cholera-like

illness, characterized by rice-water diarrhea similar to that caused by classical EL-TOR types; the disease is often associated with milder clinical manifestations (13).

The ethical approval for this study was obtained by the Scientific and Ethical Committee of the College of Pharmacy, University of Kerbala.

RESULTS

Comparison between outbreaks regarding time trend

We looked at many different characteristics of the two outbreaks in Karbala in 2007 and the two outbreaks in 2007 to see if there were any big differences. The outbreak of 2015 was occurred in period of year similar to that in the 2007 outbreak (September through November), and further-more, the 2017 outbreak was occurred during November. Table 1, shows the distribution of cholera cases according months of the years.

1. Identification Serotype of Cholera

During the 2015 outbreak, three serotypes were identified: Inaba, Ogawa, and NAG, as shown in Table 2. In contrast, only a single serotype (Inaba) was identified during the 2017 outbreak.

Table 2 shows that a statistically significant difference was observed in the number of suspected and confirmed cases between the 2015 and 2017 outbreaks ($P < 0.001$, Pearson Chi-square test at the 0.05 level of significance).

Table 1. Distribution of suspected and confirmed cholera cases during outbreaks in 2015 and 2017 in Kerbala city.

Years of Outbreak	Time	Total Number of Referred Suspected cases	Number of Confirmed Cases
2015 outbreak	From 18 September to 11 November	7609	188
2017 outbreak	From 12 to 30 November	920	76
	P value< 0.001		

Pearson Chi-square test at the 0.05 significance level.

Table 2. Serotypes of Cholera isolated from a patient's stool sample in Kerbala

Outbreak	Serotype			
	Inaba	Ogawa	NAG	Total cases from the patient (stool sample)
2015 outbreak	149	4	14	167
2017 outbreak	76	0	0	76
	P value<0.001	P value>0.05	P value<0.05	

Pearson Chi-square test at the 0.05 significance level.

2. Isolation of Cholera from water sources during the outbreaks

During both outbreaks, all water purification plants were repeatedly examined for the presence of Vibrio cholera. In addition, for each reported incident, water samples were collected from treatment plants serving the affected area.

As shown in Table 3, during the 2015 outbreak, 21 NAG isolates were recovered from water samples, whereas no isolates of other serotypes (Inaba and Ogawa) were detected. Furthermore, no Vibrio cholera isolates were recovered from any water samples collected during the 2017 outbreak.

Table 3. Isolation of Vibrio cholera from water sources (water sample)

Outbreak	Serotype		
	Inaba	Ogawa	NAG
2015 outbreak	0	0	21
2017 outbreak	0	0	0

DISCUSSION

The findings of this study highlight two key aspects for epidemiological interpretation of incidence trends in Kerbala city.

Seasonal Trends in Incidence of Cholera.

The historical analysis of cholera outbreaks in Kerbala indicates that the highest-risk period spans the autumn through early-winter months. This pattern may be attributed to several factors, including seasonal climatic changes, weather, and a lack of potable water, as annual declines in water levels in Iraq's major rivers (the Tigris and Euphrates), especially the Euphrates, which supplies water to Kerbala city. These temporal trends emphasize the importance of implementing targeted preventive strategies during high-risk periods. Furthermore, enhanced surveillance and strengthened water safety measures are especially critical during this time.

In 2015, the incidence rate was higher than that reported during the 2007 outbreak in Baghdad, where 136 cases occurred among 46,667 suspected cases (10,16). It is also the case that, when comparing the two outbreaks of the years (2015 & 2017), the 2017 cases are lower than in 2015, in agreement with Omar's report (17). This finding is consistent with significant improvement in measures related to potable water safety and the disease surveillance system implemented by the 2017 outbreak.

Effect of mass gathering events on the incidence of cholera

In Kerbala, the risk of cholera transmission is more complex because the city faces unique epidemiological challenges due to large mass gatherings associated with religious events, which can increase the risk of cholera transmission. However, during the 2015 outbreak, the final cholera cases were reported several days before the start of a major mass-gathering event in the city. Surprisingly, no cases were detected during the event itself. In contrast, the 2017 outbreak coincided with a period of mass gatherings. Indeed, all cases were identified among visitors to the city and pilgrims. The index

cases were traced to a stool sample collected from a patient originating from Pakistan.

These findings suggest that nearly all cases reported during the 2017 outbreak were directly associated with the outbreak event. By contrast, the cases reported during the 2015 outbreak may represent a combination of outbreak-related cases and sporadic annual infections. Alternatively, the 2015 outbreak may have comprised multiple concurrent outbreaks involving different serotypes.

Serotype Distribution and Environmental Reservoirs

Bacteriological investigation of all isolates in this outbreak revealed that V. cholerae biotype El Tor, serogroup O1, with 3 serotypes, was present in all isolates during 2015. This pattern differed from that of the 2007 outbreak, indicating the emergence of new serotypes in 2015 and a lack of compatibility with the 2007 outbreak. In contrast, only one serotype (Inaba) was identified during the 2017 outbreak, consistent with the 2007 outbreak (18).

Statistical analysis demonstrated that the serological pattern of Inaba and the NAG serotype of cholera were significantly associated with cholera cases, whereas Ogawa wasn't (P value < 0.05). This indicates that these two types are more prevalent in this region. Similar findings have been reported in studies conducted in Uganda and Sulaymaniyah province in Iraq (19,20). These differences may be due to genetic variation among serotypes. The differences in growth dynamics are due to enhanced environmental persistence under favorable conditions.

Apparently, the extent and occurrence of V. cholera NAG in wastewater and surface water are unknown. An extensive investigation in Iran of approximately 22,000 fecal samples found 160 positive cases (0.72%) (21). However, the asymptomatic carriers among returning pilgrims were considerably lower, approximately (8.38%).

Isolated serotypes of cholera from water sources/samples

The absence of Inaba and Ogawa isolates in water purification plant samples suggests that transmission may have occurred through alternative sources. Table 3 findings suggest that transmission of both the Inaba and Ogawa strains could occur from sources other than the local water purification plants. Indeed, potable water in Kerbala is not exclusively from the municipal supply, as many residents rely on bottled water imported from other regions. Contaminated bottled water, therefore, represents a plausible transmission route. Additionally, foodborne transmission cannot be excluded.

Hence, environmental contamination from wastewater and surface water may also play a role, as these environments can harbor V. cholerae NAG (22). Migratory birds feeding in contaminated areas may contribute to long-distance dissemination by excreting the organism. These findings indicate that V. cholerae NAG can persist in soil and aquatic

environments, highlighting the need for further investigation into the role of animal reservoirs in cholera ecology and transmission.

CONCLUSION

The results of this study demonstrate a substantial decline in cholera incidence in Kerbala city between the 2015 and 2017 outbreaks, underscoring the benefits of extensive public health efforts. Ultimately, the experience in Kerbala affirms that integrated efforts to improve water and sanitation, enhance surveillance, and mount timely responses were pivotal in disrupting transmission pathways and reducing morbidity and mortality.

In our study, we recommend continuing investment in water and sanitation infrastructure, including expanded chlorination programs, to further reduce Vibrio cholerae contamination in municipal water supplies. Additionally, strengthening epidemiological surveillance and laboratory diagnostic capacity particularly serotyping and antimicrobial susceptibility testing will enhance early detection and outbreak preparedness. The incorporation of molecular epidemiology tools into routine surveillance is also recommended to guide vaccine strategies and improve outbreak prediction.

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Conflict of Interest Statement: The authors declare that they have no conflict of interest.

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