University of Thi-Qar Journal of Science (UTJsci)
E-ISSN: 2709-0256, P-ISSN: 1991-8690, Vol. 11, No. 2, Dec. 2024

Diagnosis of Vibrio cholerae isolated from patients with Vibriosis in Thi-Qar province

Kareema Helal Shnawa Asaakrah *[a] and Hanaa Daaj Khalaf Al-Mozan[b]
Department of Biology , College of Science, University of Thi-Qar , Thi-Qar,Iraq.
[b] *E-mail: hanaa.d@sci.utq.edu.iq*
*[a] *Corresponding author: kareema.helal@utq.edu.iq*

Received: 2024-03-06 ,Revised: 2024-04-11, Accepted: 2024-04-18, Published: 2024-12-05

***Abstract*— The gram-negative bacteria *Vibrio cholerae* is the source of cholera a severe case of diarrhea. Cholera occurs either sporadically, epidemically, or endemic. Overpopulation, untreated water supplies, and unsanitary circumstances have historically been closely linked to cholera outbreaks and pandemics. This study aimed to determine the accuracy of routine and advanced biochemical methods in diagnosing *Vibrio cholerae*, the extent of the prevalence of bacteria and which age groups and gender are most susceptible to infection. Stool specimens were obtained from patients suspected with cholera disease from both gender in different hospitals in Thi-Qar province through the period from September to December 2023. Cary Blair medium was used to transfer the suspicious stool specimens to the Thi-Qar province's Central Health Laboratory. Routine and advanced biochemical methods were used to diagnose *Vibrio cholerae*. A total of 400 specimens of stool from patients suspected cholera have been collected, only (45) isolates were given growth *V. cholerae* with 11.25 % in biochemical routine test while in API20 E test 41 isolates were given growth *V. cholerae* with 10.25%. The age group with the highest infection rate was 55–65, whereas the age group with the lowest infection rate was under 5 years. With no statistically significant difference, the illness most commonly impacted females. The results have showed different morphology characteristics of all *V. cholerae* isolates which grow on different media. Routine and advanced biochemical tests may not give the same results, which have prompted to search for a more accurate and faster method of diagnosis.**

***Keywords*—Vibrio cholerae, Isolation of bacteria, Biochemical test, Diarrhea, API 20 E Vibriosis.**

I. Introduction

Vibrio is a genus of bacteria found in aquatic ecosystems in different regions of tropical and temperate climates worldwide [1] and Vibriosis is a disease caused by certain harmful and contagious Vibrio species and strains. This term describes both local and more widespread septicemic illnesses. The three most pathogenic species frequently linked to foodborne diseases are *Vibrio vulnificus*, *Vibrio parahaemolyticus*, and *Vibrio cholerae* [2].

The most important species is *Vibrio cholerae* gram-negative bacteria that causes cholera, a severe acute watery diarrheal disease caused by the O1 and O139 serogroup of *Vibrio cholerae* [3]. In every region of the world and across all age groups, diarrhea is a critical factor in morbidity and death [4].

The powerful enterotoxin known as choleragen is the cause of the deadly cholera symptoms. It is produced by all strains of *Vibrio cholerae* and is responsible for the largescale release of water and electrolytes into the intestinal lumen [5]. Every year, four million cases of cholera are reported, accounting for around 140,000 deaths [6]. *Vibrio cholerae* infects all ages and genders especially elderly people and children [7].

Early detection of infection and prompt intervention implementation across all contexts are made possible by prompt diagnosis [8]. The purity of the culture is essential to the outcome of the biochemical tests. To achieve this, certain steps must be taken, including gathering, transporting, and purifying the sample, using a selective medium, adjusting temperatures, and extending the incubation period. All of these steps cost time and effort [9]. The spread of the illness, a rise in morbidity, death rates, and inadequate public health measures are only a few of the effects of delayed diagnosis of cholera epidemics.

II. Methods

A. Isolation and diagnosis of Vibrio cholerae

1- Collection of specimens

Stool specimens were taken from both sexes of individuals who showed symptoms of watery diarrhea between September and December 2023. Fecal specimens were placed right away in a sterile container with Cary Blair medium [10].

2- Culturing of bacteria

After mixing alkaline peptone water with feces and incubating it for 6-8 hours at 37 C, we distributed a 10 µl loop full of the inoculated alkaline peptone water on TCBS, MacConkey agar, and Blood agar. Then we incubated the agar plate for 18-24 hours at 37 C, and read the plates. A gram stain was performed on suspected colonies of *V. cholerae* growing on TCBS, MacConkey agar, and Blood agar before we moved them to a non-selective medium (nutrient agar). Then, to validate the presence of *V. cholerae* in the bacterium, biochemical assays were performed [11].

https://doi.org/10.32792/utq/utjsci/v11i2.1190

B. *Biochemical test*

Biochemical methods have been used to diagnose bacteria. The important biochemical tests are Kligler iron agar, Oxidase test, Urease test, Indole test and Citrate utilization test [12].

C. *Analytical profile index for Enterobacteriaceae test (Api-20E system)*

The quick identification of *V. cholerae* isolates using the Api-20E system that is a clinical test carried out in accordance with [13].

III. RESULTS

A. *Isolation and characterization of Vibrio cholerae*

A total of 400 specimens of stool from patients suspected of cholera have been collected and tested. Only (45) isolates were given growth to *V. cholerae* with 11.25 % using biochemical test and 41 isolates with 10.25 % using API 20 E test (figure1& figure2).

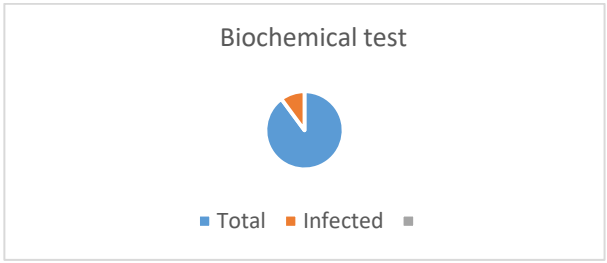

Fig. (1) percentage of *Vibrio cholerae* diagnosed by biochemical test

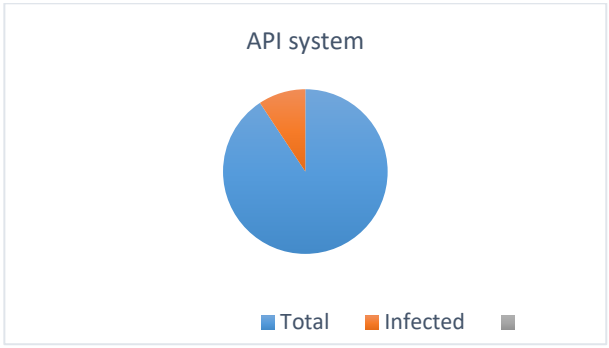

Fig. (2) percentage of *Vibrio cholerae* diagnosed by API

1. *Colony Morphology*

The results showed the different morphology characteristics of all *V. cholerae* which grow on different media (table 1& figure3).

Table (1): Culture characteristic of *Vibrio cholerae*

NO.	**Culture Media**	**Morphology of colonies**
1-	Thiosulfate citrate bile salt sucrose (TCBS)	Small,rounded,smooth,yellow colonies (Colden)
2-	Blood Agar	small, rounded, β-hemolysis
3-	MacConkey agar	Small, smooth, rounded and yellow

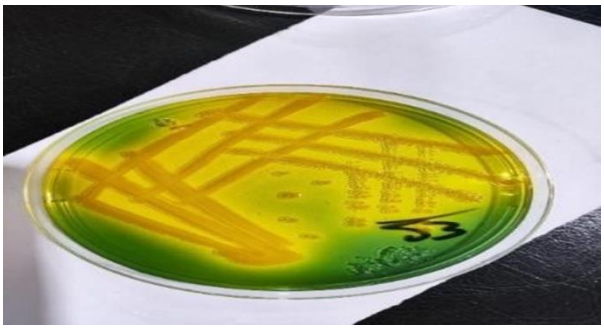

Fig. (3) Characteristic of *Vibrio cholerae* on thiosulfate citrate bile salt sucrose

2.*Biochemical test*

The results of biochemical test have revealed only 45 isolates were diagnosed as *V. cholerae* with 11.25 % (table 2,3 and figure 4).

Table (2)Result of kligler iron agar of *Vibrio cholerae*

NO	*Contents*	***Result***
1	*Slant/Bottom*	-/+
2	*H2S*	-
3	*Gas*	-

Table (3) Biochemical test of *Vibrio cholerae*

NO.	**Biochemical test**	**Result**
1	String test	+
2	Oxidase test	+
3	Indole test	+
4	Simmons citrate test	-
5	Lactose fermentation	-
6	Voges Proskauer test	+
7	Urease test	–
8	Cholera red reaction	+

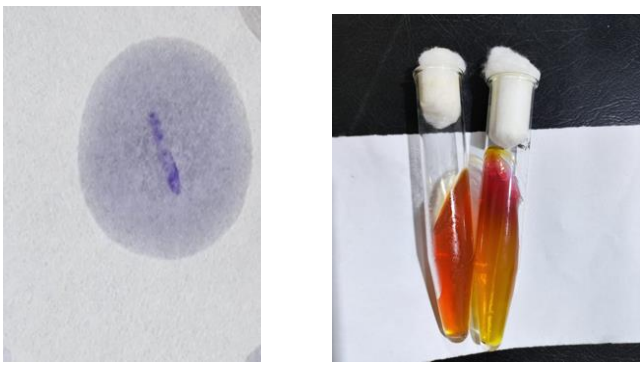

Fig. (4) Characteristic of *Vibrio cholerae* KIA in right side &oxidase test in left side

3. *API 20 E test*

The results of API-20E test have revealed that only 41 isolates from 400 specimens were identified as *V. cholerae* (table 4).

Table (4): Result of API 20E test for *Vibrio cholerae*

NO.	Biochemical test	Results
1	Arginine hydrolase(ADH)	-
2	Beta galactosidase(ONP)	+
3	ysinedecarboxylase(LDC)	-
4	Utilization Citrate (CIT)	-
5	Ornithine decarboxylase(ODC)	+
6	Production H2S (H2S)	-
7	Urease (URA)	-
8	Tryptophane Deaminas(TDA)	-
9	Indole production (IND)	+
10	Acetone production (NP)	-
11	Gelatinase (GEL)	+
12	Glucose fermentation(GLN)	+
13	Inositol fermentation (INO)	-
14	Sorbitolfermentation(SOR)	-
15	Rhamnose fermentation (RHA)	-
16	Sucrose fermentation (SAC)	+
17	Melibiose fermentation (MEL)	+
18	Amygdalin fermentation (AMY)	+
19	Arabinose fermentation (ARA)	+

B. Distribution of bacteria according to gender

The current study recorded that the infected males with bacteria were 17 (8.42%) out of 202 patients, while the infected females were 24 (12.12%) out of 198 patients. Statistical analysis at a level p. value 0.222 >0.05 has no statistically significant differences, table (5).

Table (5) Result of bacterial infection according to gender

Gender	Examined specimens	Infected specimens	Percentage%
Males	202	17	8.42%
Females	198	24	12.12%
Total	400	41	10.25%

C. Distribution of bacteria according to age groups

The present study recorded the highest percentage of (50%) in the (55-65) age group, while the lowest recorded infection (under 5 years old) with (4.55%). Statistical analysis at a level p. value which is less than 0.05, revealed statistically significant differences, table (6).

Table (6): Result of bacterial infection according to age groups

Categories	Examines	Infected	Percentage%
under 5 years old	88	4	4.55
5-15	110	9	8.18
15-25	87	14	16.09
25-35	59	4	6.78
35-45	30	3	10
45-55	14	2	14.29
55-65	8	4	50
65 and older	4	1	25
Total	400	41	10.25
Chi-square= 22.5		Df =7 p. value=<0.05	

IV. DISCUSSION

One of the main pathogens in the globe, particularly in developing nations, is *Vibrio cholerae*, which causes cholera [14]. Cholera is one of the major global health problems, especially in Africa and southern Asia; the World Health Organization estimates that it infects between 1.3 and 4 million people year and kills between 21,000 and 140,000 people globally [15]. Certain Vibrio species can be problematic because their biochemical traits vary from one species to the next [16].

When biochemical tests are not highly reliable, they might be used to diagnose bacteria inferentially rather than definitively [17]. However, biochemical tests, such as API tests, that save time and money and are more accurate than standard tests [4].

The findings of this study are consistent with the authors of [18], who discovered that there was no statistically significant difference in the disease's prevalence among females. The cause may be contaminated food and water, which do not apply to men and women equally, especially when takeout or ready-made food is around. Although these studies disagree with the authors of [19] who discovered that the proportion of sick males was higher than that of females, this might be since men, in particular, eat outside more frequently, which may have increased their exposure to several infections, including *V. cholerae*.

The study's results, which quantified the distribution of bacteria by age group, show that the age group with the lowest percentage of infected cases is that of children under five years. This is likely because young children spend most of their time at home and interact with others infrequently the authors of [20] who found the highest percentage of infected under 5 years. Various sample sizes, age groups, and environmental factors might be the cause of these variations in infection rates [21]. The prevalence of *V. cholerae* among patients may be influenced by several variables, including socioeconomic level, hygienic circumstances, health habits, and educational achievement.

The age group that is most affected by the condition, according to the results of the present study, is 55 to 65. The reason could be that older adults have a two to three times higher risk of developing symptomatic disease from infection than the general population. This could be explained by infectious dose thresholds and age-dependent [22].

This result disagreement with [23] in Lebanon who found the category between 55-65 is the least vulnerable to infection due to high health education and knowledge of dealing with disease . This study supports the findings of the authors of [24], who discovered that the age distribution of cholera patients in this study showed the greatest proportion (55–65) years old with the lowest percentage in children under the age of five, most likely as a result of feeding them with boiling water in a bottle.

For a considerable amount of time, Iraq has been involved in several internal and foreign wars, which have resulted in the nation's infrastructure deteriorating, homes being destroyed, immigration, a lack of electricity, and a shortage of clean water [25]. These elements collectively led to the cholera outbreak in Iraq.

V. CONCLUSION

Cholera outbreak is a bacterial disease caused by the gram-negative bacterium *V. cholerae* after consuming contaminated food which requires many tests and a staff with experience in diagnosis. There is no significant difference between males and females. The age group between (55-65) is most susceptible to cholera while the age group under 5 years is less susceptible to infection. The API test can be considered a diagnostic and confirmatory test for *V. cholerae*. The cholera epidemics in Iraq serve as a continual reminder of the necessity for the nation to implement sewage water treatment, proper sanitation, and clean, safe drinking water. It must also establish a disease surveillance system, and advanced molecular methods to find genetic differences to enhance basic healthcare, and increase public knowledge and preparedness to respond swiftly to any future cholera.

ACKNOWLEDGMENT

Thanks to everybody who supported us and made it possible for us to gather data for our study .

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

REFERENCES

[1] M. Duman ,N. Buján, S. Altun, L.J. Romalde, & I.B. Saticioglu, "Population genetic and evolution analysis of Vibrio isolated from Turkish fish farms". *Aquaculture, 562, 73878, 2023*

[2] V.R. Kamal, &P. Avijit, " Investigations of hemolytic activity and iron utilization sources of Vibrio alginolyticus" ATCC 17749. *Research Journal of Biotechnology Vol, 18, 1,2023*

[3] B. A. Muzembo, K. Kitahara, A. Ohno, J. Khatiwada, S. Dutta, S.I. & Miyoshi" . Vibriosis in South Asia: a systematic review and meta-analysis". *International Journalof Infectious Diseases,2024*

[4] R.M. AL-Hilali, & H.D.K Al-Mozan, H. D.K. "Isolation and identification of Gram negative bacteria that cause diarrhea". *University of Thi-Qar Journal of Science, 10(1),2023*

[5] E.H Mezal, &Z.K Hanan, Z. K," Increasing Antimicrobial Resistance Patterns of Vibrio cholerae Isolated from Diarrheal Patients Thi-Qar Province during 2015". *Al-Kufa University Journal for Biology,2017.*

[6] M.C.M Maluda,E. Johnson ,F. Robinson, M. Jikal, S.Y. Fong, M.J. Saffree, M.K. Fornace, & K. Ahmed, "The incidence, and spatial trends of cholera in Sabah over 15 years: Repeated outbreaks in coastal areas". *PLOS Global Public Health, 4(1), e0002861,2024.*

[7] M.A Echazarreta, & K.E. Klose, K. E," Vibrio flagellar synthesis". *Front Cell Infect Microbiol 9: 131, 2019.*

[8] C.N Dunmire, D. Chac, F. Chowdhury,A.I Khan, T.R. Bhuiyan, R.C LaRocque, Akter, A., Amin, M. A., Ryan, E. T., & Qadri, F. (2022). Vibrio cholerae Isolation from Frozen Vomitus and Stool Samples. *Journal of Clinical Microbiology, 60(10), e01084 22,2022.*

[9] Y. Guillaume, M. Debela, D. Slater, K. Vissieres, R. Ternier, M.F. Franke, J. B. Harris, &L.C Ivers, "Poor Sensitivity of Stool Culture Compared to Polymerase Chain Reaction in Surveillance for Vibrio cholerae in Haiti, 2018–2019". *Open Forum Infectious Diseases, 10(6), ofad301,2023*.

[10] H.D.K. Al-Moz'an, "Gene of 16S rRNA is the gold standard to diagnose bacteria". *World Journal of Current Medical and Pharmaceutical Research, 139–178, 2023*.

[11] R.F. Alaouadi, "Frequency of V. cholerae in Babylon Province". *Journal of Babylon University /Pure and Applied Sciences, 22, 2579–2590,2014*.

[12] shok KPanwar, S., Duggirala, K. S., Yadav, P., Debnath, N., Yadav, A., & Kumar, A. "Advanced diagnostic methods for identification of bacterial foodborne pathogens: Contemporary and upcoming challenges*". Critical Reviews in Biotechnology, 43(7), 982–,1000, 2023.*

[13] A. Petrie, S.E. Finkel, & J. Erbe, "Use of long-term E. coli cultures to study generation of genetic diversity & teach general microbiology laboratory skills". *The American Biology Teacher, 67(2), 87–92,2005.*

[14] S.R Dominguez, P.N. Doan, F. & Rivera-Chávez, F. "The intersection between host– pathogen interactions and metabolism during Vibrio cholerae infection". *Current Opinion in Microbiology, 77, 102421,2024.*

[15] F. Chowdhury, A.G. Ross, M.T. Islam, N.A.J. McMillan, & F. Qadri, "Diagnosis, management, and future control of cholera". *Clinical Microbiology Reviews, 35(3),e00211-21,2022.*

[16] T.H. Al-Naddawi, "Molecular Epidemiology of Vibrio cholera in Iraq during Outbreaks 2007 to 2009". *Ph. D. Thesis, College of Science, University of Baghdad, Baghdad, 2010.*

[17] C. Jesumirhewe, P.O. Ogunlowo, M. Olley, B. Springer, F. Allerberger, W. & Ruppitsch, "Accuracy of conventional identification methods used for Enterobacteriaceae isolates in three Nigerian hospitals". *PeerJ, 4, e2511,2016.*

[18] E. A. Bin-Hameed and H. A. Joban, 'Cholera Outbreak in Hadhramout, Yemen: The Epidemiological Weeks 2019', *Epidemiol. Heal. Syst. J., vol. 8, no. 1, pp. 40–46, 2021.*

[19] D. K. Sabir, Z. T. Hama, K. J. Salih, and K. G. Khidhir, 'A Molecular and Epidemiological Study of Cholera Outbreak in Sulaymaniyah Province, Iraq, in 2022', *Polish J. Microbiol., vol. 72, no. 1, pp. 39–46, 2023*

[20] H. Nasr, M. Al-Zumair, T. Al-Mahbashi, F. & Dureab, "Factors associated with the cholera outbreak in Al-Mahweet-Yemen": Analytic Study. *The Journal of Infection in Developing Countries, 18(01), 66–74, 2024.*

[21] K.Y. Loo, J.W.F. Law, L.T. H. Tan, P. Pusparajah, S.H. Wong, K.G. Chan, L.H. Lee, & V. Letchumanan, "The burden of Vibrio sp. infections–A scoping review". *Progress In Microbes & Molecular Biology, 6(1), 2023.*

[22] S. T. Hegde *et al.*, "Clinical surveillance systems obscure the true cholera infection burden in an endemic region," *Nature medicine*, vol. 30, no. 3, pp. 888-895, 2024.

[23] Y. Jamaleddine, H. Tashjian, M. Mahmoud, W. Azzam, A. Daher, & P. Salameh, "Lebanese Population Knowledge, Attitudes, and Practice Regarding Cholera Disease and their Knowledge and Attitude Regarding the Cholera Vaccine Following an Outbreak in Lebanon",2023.*researchsquare.com doi.org/10.21203/rs.3.rs-2480449/v1*

[24] T. Taher, W. S. Assi, M. A. Jalal, and F. T. R. Sarray, "Cholera outbreak in Wasit governorate, Iraq 2017," *Global Journal of Public Health Medicine*, vol. 2, no. 2, pp. 211-219, 2020.

[25] A.R.M. Al-Abbasi, & S.M Aema, "The cholera epidemic in Iraq during 2015". *TOFIQ Journal of Medical Sciences, 2(2), 27–41, 2015*