INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 19, No. 5, p. 36-41, 2021

RESEARCH PAPER OPEN ACCESS

Serological and Biochemical Based Identification of *Vibrio Cholerae* Isolated from Diarrheal Patients of Balochistan

Ashiq Hussain[1*], Muhammad Tahir Zehri[2], Abdul Sadiq[3], Muhammad Anwar Somro[4], Muhammad Kamran Taj[5], Sakina Khan[6], Umbreen Zafar[5], Nawab Khan Panezai[7], Saima Azam[5], Syeda Ayesha Ali[5]

[1]*Department of Pathology, Bolan Medical College ,Quetta Balochistan*

[2]*Department of Paeds Medicine, Bolan Medical College, Quetta Balochistan*

[3]*Department of Biochemistry Jhalawan Medical College Khuzdar, Balochistan*

[4]*Department of Surgery Bolan Medical College Quetta, Balochistan*

[5]*Center for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan*

[6]*Sardar Bahadur Khan Women University, Balochistan*

[7]*Livestock and Dairy Development Department, Balochistan, Pakistan*

Key words: *Vibrio*, Cholera, Isolation, Biochemical, Serological.

http://dx.doi.org/10.12692/ijb/19.5.36-41 Article published on November 30, 2021

Abstract

Cholera, an acute diarrheal disease is a major public health problem in many developing countries including Pakistan. *Vibrio cholerae* is a causative agent of cholera disease. Over 200 serogroups of *V. cholerae* have been identified, but only O1 and O139 cause cholera epidemics. Present study was design to assess the routine laboratory diagnosis of *V. cholerae* strains by using biochemical and compared them with the serological identification (multi-serogroups or serotype) of *V. cholerae*. Total 1776 samples were collected from suspected cholera patients at different hospitals of the Balochistan from 2018 to 2020. All isolates were examined and identified on the basis of colony characters on thiosulfate-citrate-bile salts-sucrose agar. Suspected colonies were subjected to gram staining, biochemical analysis and serological identification. Among the total samples (n=132;7.43%) were positive *V. cholerae* from stool samples, futher identification was done through culture characteristic, microscopic examination and biochemical tests. While (n=121;91.6%) confirmed by serotyping using polyvalent antisera. All isolates were serogroup O1 ogawa. However, remaining (n=11; 8.3%) isolates were non-O1/non-O139 serogroups as they did not agglutinate with the polyvalent Inaba and Ogawa antisera and there were no isolates agglutinated with antisera O139 during this study. It is concluded that fecal specimens suspected for *V. cholerae* O1 and/or O139 should be confirmed by using traditional culture-based methods suitable for the isolation and identification of *V. cholerae*.

* **Corresponding Author:** Ashiq Hussain ✉ shah73@gmail.com

Introduction

Cholera is an chronic intestinal disease with watery diarrhea accompanied by vomiting, which can cause dehydration and may lead to acidosis and problems of circulation. Unreliable treatment may lead to death very quickly (Sack *et al.*, 2004).

Cholera is caused by eating of food or drinking water contaminated with the *V. cholerae* (Azman *et al.*, 2013). *Vibrio* species are naturally found in marine environments all over the world. *V. cholerae* is able to survive and replicate in contaminated water.

Asymptomatically infected humans can also become an important reservoir for this organism in areas where *V. cholerae* is endemic (Igbinosa and Okoh, 2008).

The most authentic test for cholera validation is conducted by either stool samples or by swabs of rectum. Both of these samples is microbiologically cultured for identity and isolation of responsible bacterium. Sample from stool is cultured in particular medium in plate. The medium mostly consists of thiosulphate citrate bile salts, sucrose, or taurocholate-tellurite gelatin agar. Further enrichment in alkaline peptone water is also carried out. Dependent on the type of medium used, colonies of bacterium may be confirmed directly or may require culturing on non-selective medium.

The *V. cholerae* strain is highly motile, slightly curved, gram-negative rod. *V. cholerae* survives well in faecal specimens, if it is too late to reach the laboratory then Cary-Blair transport medium should be used for transportation (Sack *et al.*, 2004). *V. cholerae* have some different features that can be used to distinguish from members of the *Enterobacteriaceae* family. One of these characteristics is the production of the enzyme cytochrome oxidase. This enzyme oxidizes the reagent tetramethyl-pphenylene-diamine-dihydrochloride to iodophenol, a purple end product which is the basis for positive oxidase test. Mostly *V. cholerae* produces acid from the fermentation of maltose, mannitol, glucose, trehalose and sucrose but with or without gas, a feature which distinguishes them from other Vibrio species. A strain of *V. cholerae* mostly produce a mucoid string when picked from a non-selective media and mixed in a drop of 0.5% sodium deoxycholate. They metabolize ornithine and lysine but not arginine (Tarh *et al.*, 2020). The Voges-Proskauer test is used to determine whether *V. cholerae* produces acetylmethylcarbinol from glucose fermentation. If present, acetylmethylcarbbinol is converted to diacetyl in the presence of α-naphthol, strong alkaline (40% KOH), and atmospheric oxygen to form a pinkish red polymer. The *V. cholerae* classical strains are negative to vogues–proskauer test (Weil *et al.*, 2015). Therefore, the present study was designed to evaluate the routine laboratory diagnosis of *V. cholerae* strains by using biochemical and compared them with the serological identification (multi-serogroups or serotype) of *V. cholerae*.

Materials and methods

Total 1776 samples were collected with a history of untreated severe diarrhea or evidence of significant dehydration at different Hospitals of Balochistan from 2018 to 2020. All samples were collected in Cary-Blair transport medium (Oxoid, Hampshire-UK) and immediately transport to the Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB) Quetta for further process.

Processing of Samples

All samples were inoculated on selective media such as thiosulfate citrate bile salts (TCBS) agar and incubated at 37℃ for 24 hours and then identified through gram's staining.

Biochemical based identification of V. cholerae

Fourteen different biochemical and sugar fermentation tests were performed for the identification of *V. cholerae*. Such as oxidase test, catalase test, indole test, voges-proskauer test, methyl red test, citrate utilization test, glucose, mannitol, sucrose, sorbitol, trehalose, maltose, inositol and lactose. Biotyping identification, all strains resistant to polymyxin B, sheep erythrocyte haemolysis.

Serological based identification of V. cholerae

Serological identification was done by slide agglutination assay using polyvalent antisera (Murex Diagnostic Limited) for the serogroup O1 (Ogawa and Inaba) and serogroup O139 (Dienka Sieken Co. Limited, Japan). Slide agglutination test, is performed on the slide with polyvalent somatic (O) antigen and monovalent antisera for exact serotype identification.

Results

Results showed that out of total samples (n=132;7.43%) samples were positive of *V. cholerae* on the base of their morphological characteristics in TCBS agar, colonies of positive isolates were appear flat, smooth yellow, nearby 2-3 mm in diameter as shown in Fig.1.

Microscopic examination

Results showed that the bacteria were gram-negative, curved rods and comma shape as shown in Fig.2. On the other hand, biotyping identification there were no inhibitory zones around the polymyxin discs showed with any of the El Tor strains as shown in Fig.3.

Biochemical based identification of V. cholerae

Biochemical tests results showed that all isolates were positive to oxidase, catalase, indole, methyl red and citrate while showed negative urease test.

Table 1. Biochemical and sugar fermentation tests for the identification of *Vibrio cholerae*.

Biochemical tests						
Oxidase	Catalase	Indole	Voges proskauer	Methyl red	Citrate test	Urease
+ve	+ve	+ve	+ve	-ve	+ve	-ve
Sugar fermentation tests						
Glucose	Mannitol	Sucrose	Trehalose	Lactose	Sorbitol	Inositol
+ve	+ve	+ve	+ve	-ve	-ve	-ve

The positives strains were noticeable with the ability to ferment the glucose, mannitol, sucrose and trehalose but not ferment to lactose, sorbitol and inositol as shown in Table-1.

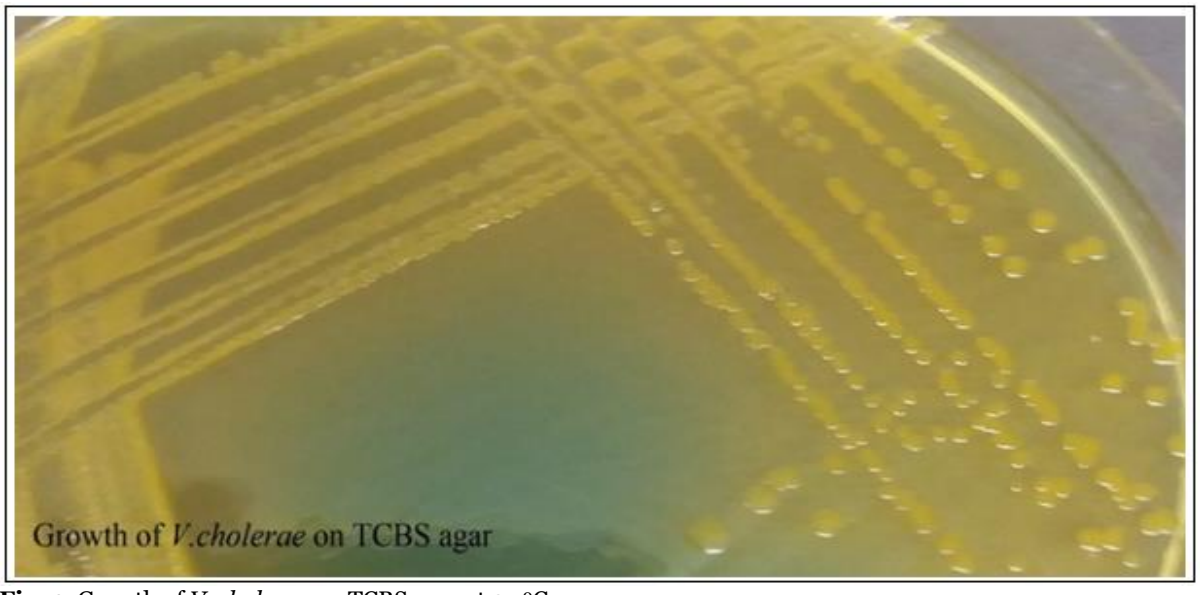

Fig. 1. Growth of *V. cholerae* on TCBS agar at 37 ^{o}C.

Serological based identification of V. cholerae

The serological test results revealed that serogroup of *V. cholerae* isolates was determined by agglutination with polyvalent antisera followed by monovalent antisera to identify the serotype. Among the total positive 132 confirmed isolates, (n=121;91.7%) were

O1 while (n=11;8.3%) isolates were non-O1/non-O139 as they did not agglutinate with the polyvalent antisera and there were no isolates agglutinated with antisera O139 during this study, as shown in Fig.4.

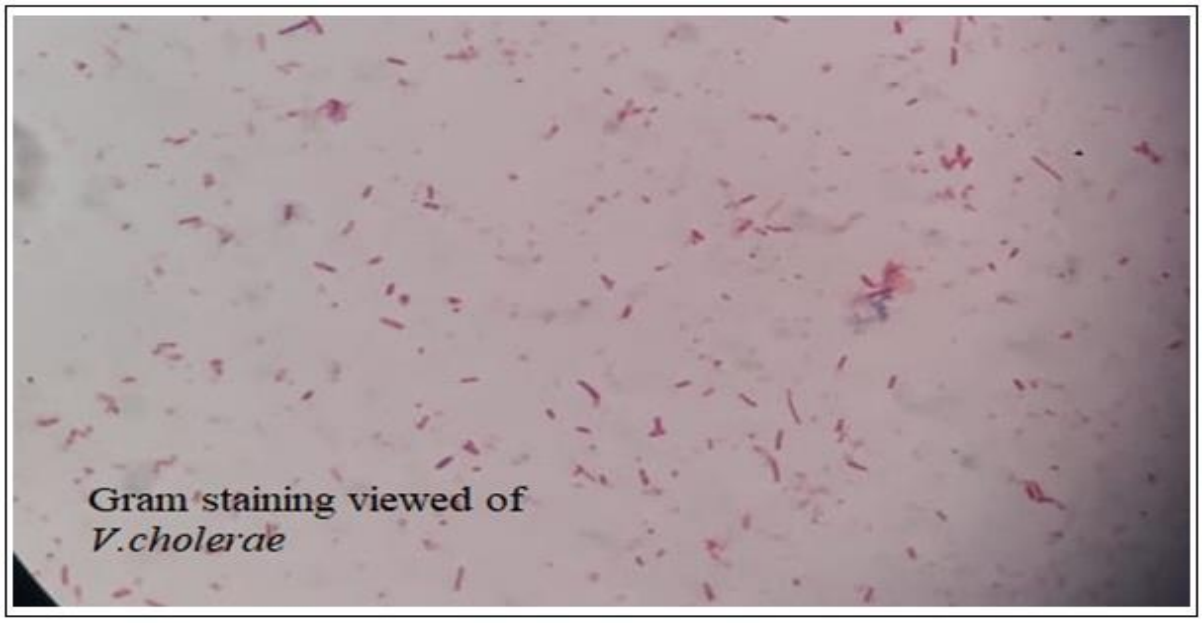

Fig. 2. Gram staining view under microscope *V. cholerae* from TCBS culture.

Discussion

Vibrio cholerae Serogroup O1, an effective cholera agent, is separated into two biotypes, classical and El Tor. Both biotypes produce major toxin. *V. cholera*e biotype ELTor, it has been a strain in the seventh global cholera epidemic. El Tour spread to Asia-Bangladesh in 1963, India in 1964 and then to the Middle East, Africa and Europe. From North Africa it spread to Italy by 1973 (Son *et al.*, 2011). El Tour infection is relatively mild, or at least fatal, and patients are asymptomatic for about a week. El Tour is more, able to survive in the body longer than the classical biotype.

This feature allows the carrier to influence the maximum population of people (Kirn *et al.,* 2005).

Fig. 3. Polymyxin B Disc resistance.

In the present study, 7.43% strains of *V. cholerae* from suspected cholera patients were isolated from all over Balochistan. Diagnosis of *V. cholerae* has been performed by isolation as well as biochemical tests similar results were founded by (Goel *et al.*, 2010).

The organism is gram-negative curved rod when stained through gram staining technique. The colony morphology of the isolates showed slightly flattened yellow colonies which are the characteristic features of the isolates on TCBS agar.

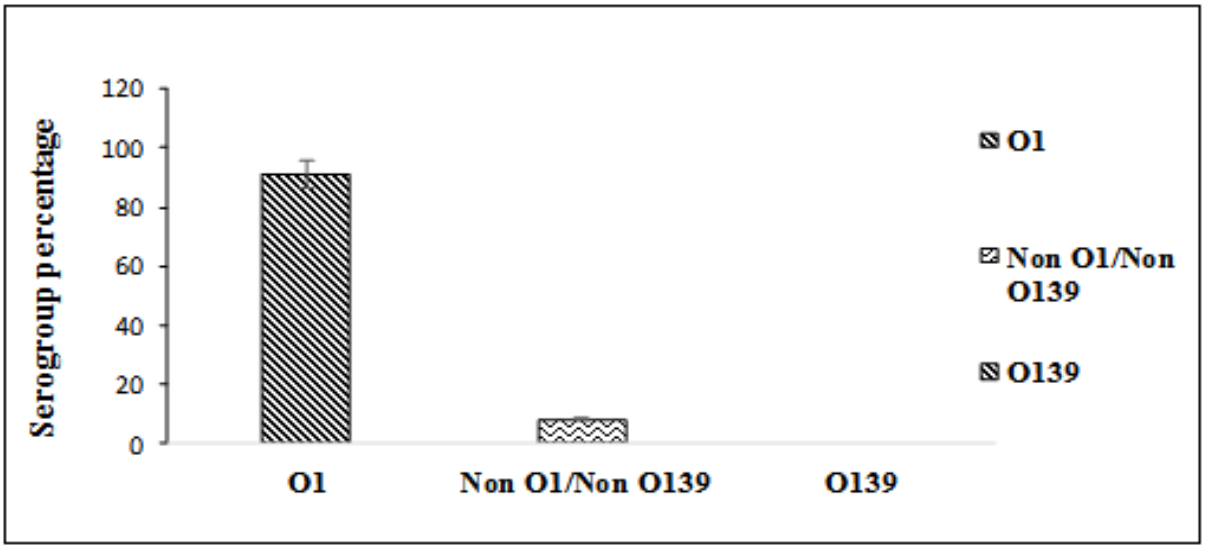

Fig. 4. Serogroup O1 and O139 of *V. cholerae*.

These findings corroborate with findings of (Sack *et al.*, 2004), they also isolated *V. cholerae* from stool samples with smooth to flattened yellow colonies to mucoid and translucent.

All the isolates were subjected to different biochemical tests such as Oxidase, Catalase, Motility, VP, Indole, Urease and Simmon citrate tests were found positive for all these isolates from stool samples of patients from all region of Balochistan. Our findings are also supported by some other researchers (Dua *et al.*, 2017: Kaper *et al.*, 1995).

These isolates fermented glucose, maltose and mannitol but did not ferment lactose, which was in accordance with the findings of Choopun *et al.*, (2002) In the current study out of 132, *V. cholerae* isolates, 91.7% were serogroup O1 while 8.3% isolates were non-O1/non-O139 serogroups. Among 91.7% serogroup O1, all isolates were belonging to Ogawa El tor biotype similar studies were founded by Rijal *et al.*, (2019); Mahanta *et al.*, (2013): Chander *et al.*, (2009) and Pirkani *et al.*, (2005). Maharjan *et al.*, (2015), reported that 22 *V. cholerae* isolates of classical biotype which was different from our current findings. Culture methods and biochemical identification of *V. cholerae* from suspected cholera may be helpful in arranging an effective preventive technique as well as effective antibiotic treatment.

Biochemical and isolation technique applied in this research may be supportive for primary diagnosis, PCR test toward the final conclusive diagnosis are required.

Conclusion

It is conclude that *V. cholerae* O1 persist in developing country and is still causing incidence of cholera. Serological identification is a good tool for identification of *V. cholerae* O1. Therefore, it is recommended that fecal specimens suspected for *V. cholerae* O1 and/or O139 should be confirmed by using traditional culture-based methods suitable for the isolation and identification of *V. cholerae*. In this study, commonly believed methods for isolation, detection, and characterization of *V. cholerae*, provided more extensive knowledge of the epidemiology of *V. cholerae*.

References

Azman AS, Rudolph KE, Cummings DA, Lessler J. 2013. The incubation period of cholera: a systematic review. Journal of Infection **66,** 432-438.

Igbinosa EO, Okoh AI. 2008. Emerging *Vibrio* species: an unending threat to public health in developing countries. Research in Microbiology **159,** 495-506.

Sack DA, Sack RB, Nair GB, Siddique AK. 2004. Cholera. Lancet **363,** 223–233.

Tarh JE. 2020. A Review on diagnostic methods for the identification of *Vibrio cholerae*. Journal of Advances in Medicine and Medical Research 136-164.

Weil A, Midani F, Chowdhury F, Khan A, Begum Y, Charles R, Larocque R. 2015. The gut microbiome and susceptibility to *Vibrio cholerae* infection. In Open Forum Infectious Diseases. Infectious Diseases Society of America **2,** 697.

Son MS, Megli CJ, Kovacikova G, Qadri F, Taylor RK. 2011. Characterization of *Vibrio cholerae* O1 El Tor biotype variant clinical isolates from Bangladesh and Haiti, including a molecular genetic analysis of virulence genes. Journal of Clinical Microbiology **49,** 3739-3749.

Kirn TJ, Jude BA, Taylor RK. 2005. A colonization factor links *Vibrio cholerae* environmental survival and human infection. Nature **438,** 863-866.

Goel AK, Jain M, Kumar P, Jiang SC. 2010. Molecular characterization of *Vibrio cholerae* outbreak strains with altered El Tor biotype from southern India. World Journal of Microbiology and Biotechnology **26,** 281-287.

Kaper JB, Morris JR, JG, Levine MM. 1995. Cholera. Clinical Microbiology Reviews **8,** 48-86.

Dua p, Karmakar A, Dutta K, Ghosh C. 2017. A simple procedure for isolation, identification and characterization of *Vibrio cholerae* from clinical samples. International Journal of Pharma and Bio Sciences **8,** 57-64.

Choopun N, Louis V, Huq A, Colwell RR. 2002. Simple procedure for rapid identification of *Vibrio cholerae* from the aquatic environment. Applied and Environmental Microbiology **68,** 995-998.

Rijal N, Acharya J, Adhikari S, Upadhaya BP, Shakya G, Kansakar P, Rajbhandari P. 2019. Changing epidemiology and antimicrobial resistance in *Vibrio cholerae*: AMR surveillance findings (2006–2016) from Nepal. BMC Infectious Diseases **19,** 1-8.

Mahanta BN, Mahanta TG, Sinha R, Dutta A, Payeng D, Jawed Q. 2013. Investigation of a cholera outbreak in a tea garden of Sivasagar district of Assam. Indian Journal of Community Medicine **38,** 240-243.

Maharjan R, Shrestha D, Acharya J, Adhikari N, Rijal KR, Ghimire P, Shrestha UT. 2015. Change in biotype trend of Vibrio cholerae in Nepal. Med-Micro Journal of Microbiology **1,** 46-54.

Pirkani GS, Iqbal M, Mohammad BA, Jogezai EK, Rasool G, Ud din N. 2005. Water born outbreak of *Vibrio cholerae* o1 ogawa. The Professional Medical Journal **12,** 247-250.

Chander J, Kaistha N, Gupta V, Mehta M, Singla N, Deep A, Sarkar BL. 2009. Epidemiology & antibiograms of *Vibrio cholerae* isolates from a tertiary care hospital in Chandigarh, north India. Indian Journal of Medical Research **129,** 613-617.