



RESEARCH PAPER

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The effect of different environmental factor temperature and PH on growth of *V. Cholerae* isolated from clinical samples in Balochistan.

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Article published on February 12, 2022

Key words: Cholerae, Temperature, pH, Media, Alkaline

Abstract

Vibrio are defined as motile, curved rod, asporogenous facultative anaerobic and gram-negative bacteria. *V. cholerae* ferment glucose and producing the oxidase and catalase without making gas. Either need NaCl for their growth stimulated by its addition. This enteric pathogen naturally inhabits an aquatic environment and can cause infections in humans. The present study was design to check the influence of temperature and pH on growth of *V. cholerae*. A distinct effect on the multiplication of *V. cholerae* was confirmed by measuring culturable counts on agar plates. Results showed that the *V. cholerae* growth was extremely depended on temperature. Best growth was observed at optimum temperature that was 30°C and 37°C, whereas less growth was observed at 20°C and no growth was observed at 15°C and above 45°C. This study revealed that best pH for *V. cholerae* growth was 10-12 while no growth was observed on 4 pH. *V.cholerae* can multiply freely in water and bacterial growth increases in contaminated waters during the warm months. At higher temperature above 40°C and below 15°C no growth was seen. Similarly, the organism exhibited best growth between pH 8-11. However, minimal growth was seen at pH 6 and pH 12. The temperature 30-37°C, and pH 8-11 may be recommended for the best growth of the *V. cholerae*.

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Introduction

Vibrio are defined as motile, curved rod, asporogenous facultative anaerobic and gram-negative bacteria. *V. cholerae* ferment glucose and producing the oxidase and catalase without making gas. Either need NaCl for their growth stimulated by its addition. Sometimes microorganisms are provided with all kinds of nourishment and required nutrition but still they may not grow well, as they may need environmental conditions. Numerous components influence on a microorganism to grow, for example, temperatures, pH and osmotic pressure. Bacterial growth at generation time and lag phase is significantly impacted by pH, acidity and alkalinity which influence every living cell (Zhalnina *et al.*, 2015). Growth of microorganism can be affected by the pH. The idea depends on when pH is transformed, it can influence catalysts which change the ionization condition of components (Amend *et al.*, 2013). Different pH limits can restrict the growth of infectious microorganisms, which have been reported through consecutive studies (Bethke *et al.*, 2011). Most bacteria are neutrophiles, it means the best and fast growth is found at neutral pH and very limited and slow growth is observed at acidic and basic pH. *V. cholerae*, the pathogenic agent of cholera, grow best at pH between 8.0 and 10.5. it can survive pH values of 11.0 but is inactivated by the acid of the stomach. Those pathogens causing disease in stomach must survive the gastric barrier, resist bile in the small intestine, and colonize the intestinal lumen (Drasar *et al.*, 1969). The different temperatures have deep effects on the structure and physical properties of including both sporulated and non-sporulated microbes, with membranes ribosomes, protein and enzymes all can be affected (Russell *et al.*, 2003).

Microorganism may grow across a wide range of temperatures, and that grow from very cold to very hot. *Vibrios* are able to grow over a wide temperature range 20°C to 40°C while microbe is found in a variety of aquatic systems, salinity and nutrient concentrations affect the development and viability of *V. cholerae*, and the results of such studies may, in part, explain the seemingly selective distribution of *V. cholerae* in estuarine system (Holmgren *et al.*, 1973; Kaper *et al.*, 1979).

The best media is that which delivers shorter log phase and higher production. For *V. cholerae* isolation there are three conventionally selective media which are thiosulfate citrate bile salts sucrose (TCBS) agar, tellurite taurocholate gelatin agar (TTGA), and CHRO Magar. The TCBS agar is the selective agar medium for the isolation of *V. cholerae* as well as other *Vibrio* species and is widely used worldwide (McCormack *et al.*, 1974). Therefore, the present study was design to check the influence of temperature and pH on growth of *V. cholerae*.

Material and methods

A total of 132 positive isolates were found during two-year study. The identification of the isolates of *V. cholerae* were enriched in alkaline peptone water for 6h and then streaked on thiosulfate citrate bile salts sucrose (TCBS) agar (Oxoid Ltd, Hampshire, UK) plates and incubated for 24h at 37°C. Typical golden yellow colonies were identified by biochemical reaction and serotyping.

Different temperatures effect on V. cholerae growth

To determine the effect of temperature, the positive sample of *V. cholerae*, were incubated for their growth at different temperatures of 15°C, 20°C, 25°C, 30°C, 35°C, 37°C, 40°C, 45°C and 50°C for 24 hrs.

Different pH effects on V. cholerae growth

The effect of pH on the growth of *V. cholerae* was determined by making a series of pH values, 4,6,8,10 and 12 with basal 4% NaOH and 1M HCl followed by autoclavation and plating. The presence or absence of colony growth on TCBS agar plate after incubation at 37°C for 24 hours was observed.

Results

Growth of Vibrio Cholerae on different temperatures:

The effect of temperature on multiplication of *V. cholerae* after 24 hours was observed and a significant difference was noted at different temperature treatment. The *V. cholerae* growth was extremely depended on temperature. High temperature did not allow *V. cholerae* to grow on

media, while the best growth of *V. cholerae* was observed on 25°C and 37°C, whereas less growth was observed on 20°C and 40°C and no growth was observed at 15°C, 45°C and 50°C, as shown in Table-1.

Table 1. Growth of *V. cholerae* on different temperatures.

SN	Temperatures (°C)	Growth	Remarks
1	15	--	No growth
2	20	+	Less growth
3	25	++	Moderate growth
4	30	+++	Good growth
5	37	++++	Extra ordinary growth
6	40	+	less growth
7	45	--	No growth
8	50	--	No growth

No growth was occurred at 15°C while less growth of *V. cholerae* was observed at 20°C and moderate growth of *V. cholerae* was noted at 25°C whereas good growth was observed at 30°C incubation as shown in Fig-1. Extra ordinary growth was observed at 37°C while less growth was noted at 40°C whereas no growth was observed at 45°C and 50°C after 24 hours' incubation as shown in Fig-2.

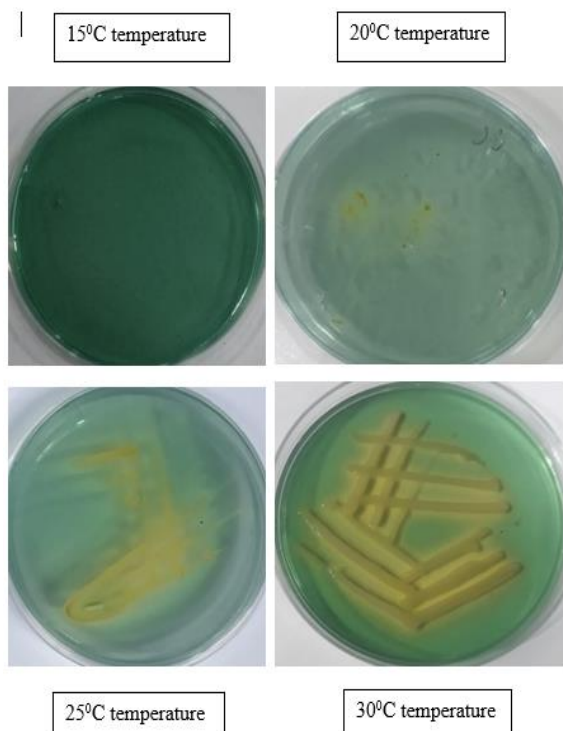


Fig. 1. Growth of *V. Cholerae* on different temperatures.

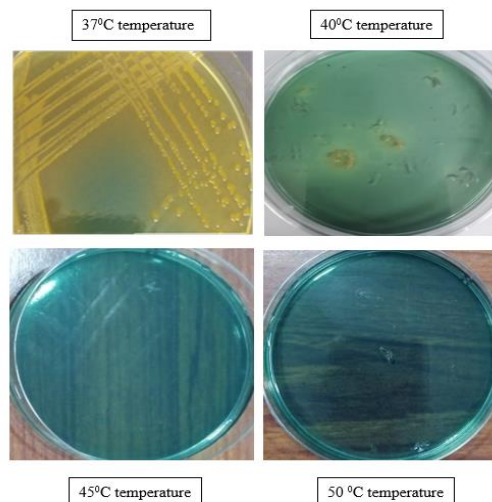


Fig. 2. Growth of *V. Cholerae* on different temperatures.

Vibrio cholerae growth on different pH:

Effect of different pH on the growth of *V. cholerae* in a laboratory environment revealed that isolates of *V. cholerae* grow in alkaline pH. The best pH for *V. cholerae* growth was 10-12 while no growth was observed at pH 4 whereas less growth and moderate growth were observed at pH 6 and pH 8 as shown in Table-2 and Fig- 3.

Table 2. Effects of pH on *Vibrio cholerae* growth.

SN	pH	Growth	Remarks
1	4	--	No growth
2	6	+	Less growth
3	8	++	Moderate growth
4	10	+++	Good growth
5	12	++++	Extra ordinary growth

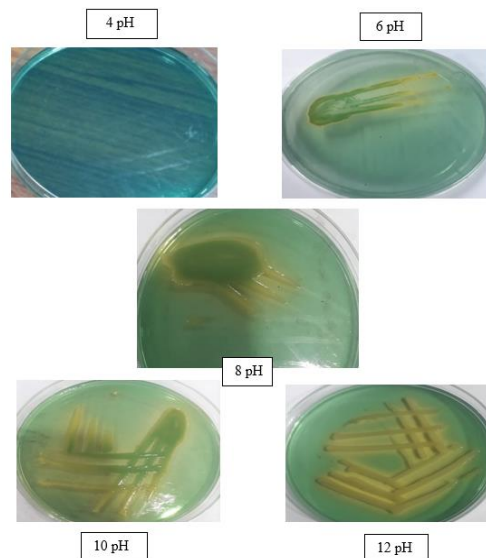


Fig. 3. Effects of pH on *Vibrio cholerae* growth.

Discussion

V. cholerae is found in marine environment all over the world and has been documented as an important clinically organism for over a century, but surprisingly, slight is recognized about its ecology, particularly its relations with environmental parameters. *Vibrio* species grow naturally in estuarine and marine environments worldwide, and are able to survive and replicate in contaminated water with increased salinity. Asymptomatically infected humans can also be an important reservoir for this organism in areas where *V. cholerae* disease is endemic (Igbinsosa *et al.*, 2008). Cholera is often described as the classic water-borne disease because it is commonly associated with water. For more developed countries, contaminated food (especially undercooked (seafood) is the usual vehicle for transmission (Sack *et al.*, 2004).

Isolated organisms were observed at different temperatures in the current study from 15 to 50°C. The organism showed best growth at 37°C. However, growth was also seen at 20-40°C, but no growth was seen at 15°C, 45°C and 50°C. Our findings are in line with, AL-Hadrawi and Ahmed, (2018), who also evaluated the *V. cholera* growth at different temperatures and reported 30-37°C to be the best and optimal temperature for the growth of tested *V. cholerae* strains.

The pH trial showed maximum growth of *V. cholerae* between 8 -11 as compared with low pH. This observation is almost similar to the reported by Kiiyukia *et al.*, (1993); AL-Hadrawi and Ahmed, (2018) findings.

Conclusion

V.cholerae can multiply freely in water and bacterial growth increases in contaminated waters during the warm months. At higher temperature above 40°C and below 15°C no growth was seen. Similarly, the organism exhibited best growth between pH 8-11. However, minimal growth was seen at pH 6 and pH 12. The temperature 30-37°C, and pH 8-11 may be recommended for the best growth of the *V. cholerae*.

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