

SHORT REPORT

Survival of *Shigella flexneri* in Artificial Aquatic Environment: Effects of Different Physicochemical Stress Factors

M SIRAJUL ISLAM¹, FERDOUSI BINTE REZWAN², AND SIRAJUL ISLAM KHAN²

¹Laboratory Sciences Division, International Centre for Diarrhoeal Disease Research, Mohakhali, Dhaka 1212;
²Department of Microbiology, University of Dhaka, Ramna, Dhaka 1000; Bangladesh

ABSTRACT

Survival of bacteria in aquatic environment is influenced by the prevailing physicochemical characteristics of water. A study was carried out to assess the effects of temperature, pH, and salinity of water on the survival of *Shigella flexneri* in laboratory microcosms. Survival of *S. flexneri* was assessed by viable counts of *Shigella* on MacConkey agar plates. At pH 7.0 and temperature of 4 °C, *S. flexneri* survived longer in 0.5% salinity than in salt-free distilled water, while at pH 7.0 and at higher temperatures (25 °C and 37 °C), the bacteria survived longer in distilled water than in 0.5% salinity. It was also observed that 2.0% NaCl solution was detrimental to bacterial survival at pH 8.0 and temperature of 37 °C. Survival times of the viable cells increased with reduction of temperatures and salinity. These findings demonstrated that survival of *S. flexneri* in the aquatic environment is greatly influenced by physicochemical factors.

Key words: *Shigella flexneri*; Dysentery, Bacillary; Water microbiology

INTRODUCTION

Shigellosis is endemic in Bangladesh and occasionally causes epidemic outbreaks (3). It is an important cause of mortality in the country, particularly in malnourished children (14).

Contaminated water is regarded as an important vehicle for transmission of the infection (1). In the United States, outbreaks of shigellosis were attributed to swimming in contaminated water. In July 1969, 37 children became ill after swimming in a wading pool in Oregon, USA and in August 1974, 31 cases of shigellosis in Dubuque, Iowa, were traced to swimming in Mississippi river water (20).

The epidemiological behaviour of an organism, especially bacteria, depends mainly on how the organism adapts to the environment or the host. The epidemio-

logical studies of shigellosis in Bangladesh have shown that surface water, e.g. ponds, lakes, wells, and rivers, can act as sources of infection (14, 19). Therefore, it would be useful to study the organism's physiological properties that allow it to survive in environmental waters.

In the past, limited studies were carried out with *Shigella* in microcosms using nutrient agar and 5% blood agar slant using some non-standardized procedures (12,19). However, studies on the impact of combinations of various physicochemical parameters on the survival of *S. flexneri* using standard procedures are limited. Therefore, the present study was undertaken to investigate the impact of various physicochemical conditions of water on the survival of *Shigella flexneri* in laboratory microcosms using standard procedures.

Correspondence and reprint requests should be addressed to:
Dr. M Sirajul Islam

MATERIALS AND METHODS

Bacterial strain

One strain of *S. flexneri* (No.16455), obtained from the Clinical Microbiology Laboratory of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka, was used in this study. This strain was identified by morphological, biochemical, and serological tests following standard procedures (10, 11).

Physicochemical factors

Temperatures of 4 °C, 25 °C, and 37 °C, salt (NaCl) concentrations of 0.5%, 1.0%, and 2.0%, and pH of 6.0, 7.0 and 8.0 were studied.

Preparation of inoculum

The strain was inoculated onto a MacConkey agar (Difco, USA) plate and incubated at 37 °C for 24 hours. A loopful of the growth was resuspended in tubes containing 10 ml of 0%, 0.5%, 1.0%, and 2.0% NaCl solutions. The optical transmittance of the suspension was determined at 585 nm wave length using a spectrophotometer (Coleman, Junior IIA, Model 6/20A, USA), and the concentration of the cell suspension was adjusted to the desired levels. Then the number of cells/mL was assessed by viable counts on MacConkey agar using the drop plate technique.

Preparation and inoculation of suspending fluid and storage of bottles

In a 500-mL conical glass flask (Pyrex), 100 mL of suspending fluid was placed. Then a measured inoculum of about 10^5 organisms/mL of *S. flexneri* was added and mixed. Suspending fluids of various salinities (0%, 0.5%, 1.0%, and 2.0%) were prepared in distilled water and adjusted to pH 6.0, 7.0, and 8.0 by adding 0.1 N HCl or 0.1 N NaOH as required. The flasks were then stored at various temperatures (4 °C, 25 °C, and 37 °C).

Sampling time and counting procedures

The viable cells were counted at 0, 2, 4, 6, and 24 hours and at different time intervals until the viable cells were absent. The counting of any suspension was discontinued after failure to recover *S. flexneri* on two consecutive samplings. At each time, 0.1 mL sample from each flask was taken, and 10-fold dilutions were prepared in phosphate-buffered saline (PBS), and then 25 µl from different dilutions were plated on MacConkey agar plates. The plates were then incubated at 37 °C for 24 hours. Bacterial counts were derived from counts of individual colonies and expressed as colony-forming units (cfu)/mL.

RESULTS

Fig. 1 shows the survival of *S. flexneri* at three different pH levels (6.0, 7.0, and 8.0) at three different temperatures (4 °C, 25 °C, and 37 °C) at 0.5% salinity. *S. flexneri* survived up to day 34, 39 and 18 at pH 6.0, 7.0 and 8.0 respectively at 4 °C; on the other hand, at 25 °C the cells survived up to 16, 15 and 5 days at pH 6.0, 7.0 and 8.0 respectively. In case of 37 °C, the cells survived up to 24 hours both at pH 7.0 and pH 8.0 and 4 days at pH 6.0. At lower temperature, i.e. at 4 °C, neutral pH was better than acidic and alkaline pH, but at higher temperatures, i.e. 25 and 37 °C, pH 6.0 supported better survival when compared with other pH levels.

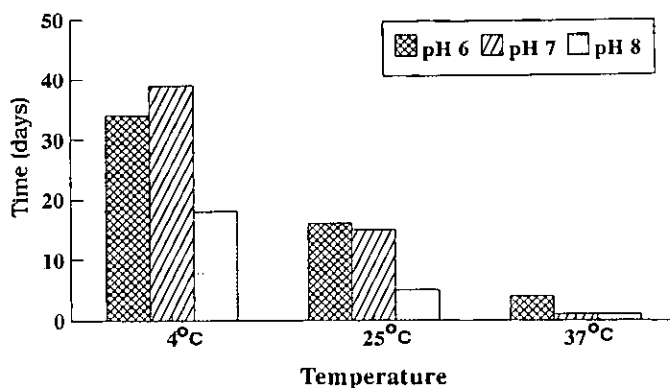


Fig. 1. Survival of *S. flexneri* at different temperatures and pH levels at 0.5% salinity

Fig. 2 depicts the survival of *S. flexneri* at 1.0% NaCl concentration. *S. flexneri* survived up to 32, 34, and 10 days at pH 6.0, 7.0, and 8.0 respectively at 4 °C, whereas the cells survived up to 8, 13, and 5 days at pH 6.0, 7.0, and 8.0 respectively at 25 °C. At 37 °C, *S. flexneri* could survive up to 24 hours at three different pH levels and could not be isolated on day 2. The results indicate that pH 7.0 was better than the other pH levels at 4 °C.

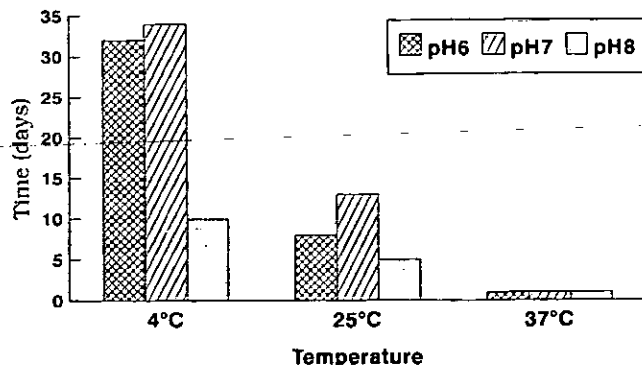


Fig. 2. Survival of *S. flexneri* at different temperatures and pH levels at 1.0% salinity

Fig. 3 represents the survival of *S. flexneri* at 2.0% salinity. At 37 °C, the cells survived up to 24 hours at pH 6.0 and 7.0 and 6 hours at pH 8.0. The strain survived up to 8, 9, and 2 days at pH 6.0, 7.0, and 8.0 respectively at 25 °C, whereas at 4 °C the cells survived for 22, 16, and 8 days at pH 6.0, 7.0, and 8.0 respectively. It was observed that at 2.0% salinity, pH 6.0 was relatively better than pH 7.0 and 8.0, at 4 °C temperature.

Fig. 4 describes the comparative survival of *S. flexneri* in control water at three different pH at three different temperatures. The effect of pH and temperatures on the survival of *S. flexneri* in control water demonstrated that the organism survived longer at neutral pH than alkaline or acidic pH in all the temperatures examined. However, pH 7.0 was better than pH 6.0 and 8.0 for the survival of *S. flexneri* at 0% salinity. It was also observed that with the increase of temperature, the survival time decreased, irrespective of pH levels.

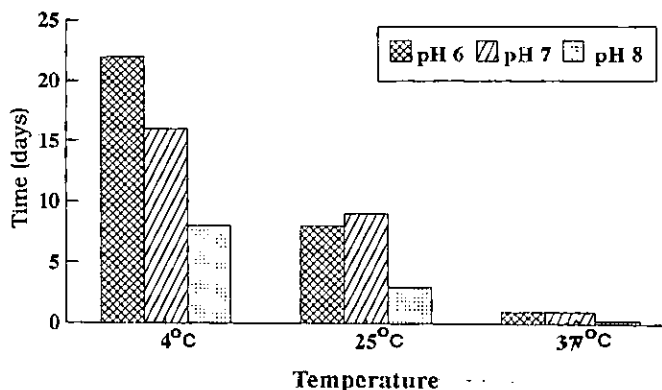


Fig. 3. Survival of *S. flexneri* at different temperatures and pH levels at 2.0% salinity

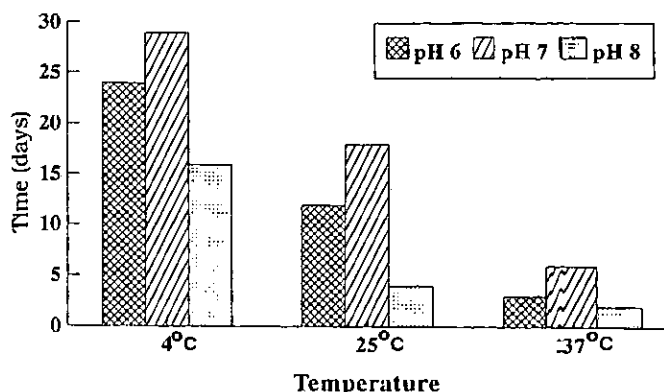


Fig. 4. Survival of *S. flexneri* at different temperatures and pH levels at 0% salinity

DISCUSSION

The present study demonstrated the impact of various physicochemical conditions of water on the survival of *S. flexneri*. Among the three temperatures tested, lower

temperature (4 °C) was found to be suitable for the longer survival of *S. flexneri*, irrespective of salinities and pH levels examined. Therefore, if water contaminated with low number of *S. flexneri* kept in a refrigerator, they can survive 8-39 days and provide a potential source of infection, since the infective dose of shigellosis is very low. It has been demonstrated that ingestion of 10-100 bacteria can cause the disease in humans (15).

Miller *et al.* (16) in a study on the response of toxigenic *Vibrio cholerae* O1 to physicochemical stresses in an artificial aquatic environment observed that the optimal pH range for the survival of *V. cholerae* was 7.5-9.0 at 25 °C at low salinity (0.05%). In the present study, it was found that in water without NaCl solution, pH 7.0 was the optimum for the survival of *S. flexneri* at any of the temperatures examined. The pH of potable water is around 7 which can support better survival of *S. flexneri* and may help in the transmission of the disease.

S. flexneri is the predominant serotype of *Shigella* in Bangladesh (14). It has been demonstrated that the improvement of water supply and sanitation can considerably reduce the incidence of shigellosis in Bangladesh (13). It was also shown that use of open sources of water, e.g. ponds, lakes, and rivers, was a significant factor for contracting shigellosis (14). Further, the experimental findings in this study, which show that *Shigella* survives for a long period in water, are other explanations of the importance of water as the reservoir of *Shigella* in the environment.

The present study demonstrated that the survival of *S. flexneri* in the aquatic environment is greatly influenced by the prevailing physicochemical factors, including the temperature, pH, and salinity. Oppenheimer *et al.* (18) showed that the average temperature, pH, and salinity in fresh water environment of Dhaka, Bangladesh are 26 °C, 7.2, and 0.004% respectively which are close to our experimental conditions (e.g. 25 °C, pH 7.0 and 0% salinity). In these conditions, *S. flexneri* survived more than two weeks. Therefore, these findings give an insight into the mechanism of adaptation of *S. flexneri* in the aquatic environment in Bangladesh. This study indicates the probable role of the water sources, e.g. ponds, lakes, rivers, etc., on transmission of shigellosis. People may contract shigellosis during interaction with these water bodies during bathing, swimming, washing, and drinking.

ACKNOWLEDGEMENTS

This research was supported by the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The ICDDR,B is supported by countries and agencies which share its concern for the health problems of developing countries. Current donors include: the aid agencies of the Governments of Australia, Bangladesh, Belgium, Canada, Denmark, Germany, Japan, the Netherlands, Norway, Saudi Arabia, Sri Lanka, Sweden, Switzerland, Thailand, the United Kingdom

and the United States; international organizations including Arab Gulf Fund, Asian Development Bank, European Union, the United Nations Children's Fund (UNICEF), the United Nations Development Programme (UNDP), the United Nations Population Fund (UNFPA), and the World Health Organization (WHO); private foundations including Aga Khan Foundation, Child Health Foundation (CHF), Ford Foundation, Population Council, Rockefeller Foundation and the Sasakawa Foundation; and private organizations including American Express Bank, Bayer A.G., CARE, Family Health International, Helen Keller International, the Johns Hopkins University, Macro International, New England Medical Centre, Procter Gamble, RAND Corporation, SANDOZ, Swiss Red Cross, the University of Alabama at Birmingham, the University of Iowa, Wander A.G. and others.

The authors are grateful to Prof. RB Sack and Dr. A Hossain for reviewing the manuscript and Mr. Manzurul Haque for typing the manuscript.

REFERENCES

1. Barrell RAE, Rowland MGM. Infant foods as a potential source of diarrhoeal illness in rural West Africa. *Trans R Soc Trop Med Hyg* 1979;73:85-90.
2. Colwell RR, Singleton FL, Huq A, Xu HS, Roberts N. Ecology of *Vibrio cholerae*, *Vibrio parahaemolyticus* and related vibrios in the natural environment. In: Takeda Y, Miwatani T, Editors. Bacterial diarrheal diseases. Tokyo: KTK Scientific Publishers, 1985:273-95.
3. Hossain MA, Albert MJ, Hasan KZ. Epidemiology of shigellosis in Teknaf, a coastal area of Bangladesh: a 10-year survey. *Epidemiol Infect* 1990;105:41-50.
4. Huq A, West PA, Small EB, Huq MI, Colwell RR. Influence of water, temperature, salinity and pH on survival and growth of toxigenic *V. cholerae* serovar O1 associated with live copepod in laboratory microcosm. *Appl Environ Microbiol* 1984;48:420-4.
5. Islam MS. Effect of various biophysicochemical conditions on toxigenicity of *Vibrio cholerae* O1 during survival with a green alga, *Rhizoclonium fontanum*, in an artificial aquatic environment. *Can J Microbiol* 1990;36:464-8.
6. Islam MS. Increased toxin production by *Vibrio cholerae* O1 during survival with a green alga, *Rhizoclonium fontanum*, in an artificial aquatic environment. *Microbiol Immunol* 1990;34:557-63.
7. Islam MS, Drasar BS, Bradley DJ. Long term persistence of toxigenic *Vibrio cholerae* O1 in the mucilaginous sheath of a blue green alga, *Anabaena variabilis*. *J Trop Hyg Med* 1990;93:133-9.
8. Islam MS, Drasar BS, Bradley DJ. Survival of toxigenic *Vibrio cholerae* O1 with a common duckweed, *Lemna minor*, in artificial aquatic ecosystems. *Trans R Soc Trop Med Hyg* 1990;84:422-4.
9. Islam MS. Toxigenicity and toxin genes of *Vibrio cholerae* O1 isolated from an artificial aquatic ecosystem. *World J Microbiol Biotechnol* 1991;7:269-71.
10. Islam MS, Hasan MK, Khan SI. Growth and survival of *Shigella flexneri* in common Bangladeshi foods under various conditions of time and temperature. *Appl Environ Microbiol* 1993;59:652-4.
11. Islam MS, Hasan MK, Miah MA et al. Use of the polymerase chain reaction and fluorescent antibody methods for detecting viable but non-culturable *Shigella dysenteriae* type 1 in laboratory microcosms. *Appl Environ Microbiol* 1993;59:536-40.
12. Jameison W, Madri P, Clans G. Survival of certain pathogenic microorganisms in sea water. *Hydrobiologia* 1976;50:117-21.
13. Khan MU. Interruption of shigellosis by hand washing. *Trans R Soc Trop Med Hyg* 1982;76:164-8.
14. Khan M, Curdin GT, Huq I. Epidemiology of *Shigella dysenteriae* type 1 infections, in Dacca urban area. *Trop Geogr Med* 1979;31:213-23.
15. Levine MM, DuPoint HL, Formal SB et al. Pathogenesis of *Shigella dysenteriae* 1 (Shiga) dysentery. *J Infect Dis* 1973;127:261-70.
16. Miller CJ, Drasar BS, Feachem RG. Response of toxigenic *Vibrio cholerae* O1 to physicochemical stresses in aquatic environments. *J Hyg Camb* 1984;93:475-95.
17. Mohadjer S, Mehrabian S. Studies on the survival of *Shigella flexneri* in river and tap water. *Arch Roum Path Exp Microbiol* 1975;34:307-12.
18. Oppenheimer JR, Ahmed MG, Huq A. Limnological studies of three ponds in Dacca, Bangladesh. *Bangladesh J Fish* 1978;1:1-28.
19. Rahaman MM, Khan MM, Aziz KMS, Islam MS, Kibria AKMG. An outbreak of dysentery caused by *Shigella dysenteriae* type 1 on a coral island in the Bay of Bengal. *J Infect Dis* 1975;132:15-9.
20. Rosenberg ML, Hazlet KK, Schaefer J, Wells JG, Pruneda RC. Shigellosis from swimming. *J Am Med Assoc* 1976;236:1849-52.