TÜBİTAK

ORIGINAL ARTICLE

Turk J Med Sci
2008; 38 (4): 307-310
© TÜBİTAK
E-mail: medsci@tubitak.gov.tr

Survival of *Salmonella typhi* and *Shigella flexneri* in Different Water Samples and at Different Temperatures

M. Hamidullah UYANIK
Halil YAZGI
Ahmet AYYILDIZ

Department of Microbiology and Clinical Microbiology, Faculty of Medicine, Atatürk University, Erzurum - TURKEY

Aim: In this study, we aimed to evaluate the survival of *Salmonella typhi* and *Shigella flexneri*, which have significance for public health, in various aqueous media (distilled water, 0.9% NaCl solution, chlorinated water) and at different temperatures.

Materials and Methods: The water samples mentioned above were contaminated with approximately 5×10^5 bacteria/ml of *S. typhi* and *S. flexneri*, and then stored at 37 ºC, room temperature, in refrigerator (4 to 6 ºC) and in deep freezer (-20 ºC). From the water materials prepared accordingly, cultures were done beginning in the first 24 hours and later every other day for viability control of the bacteria until no growth was detected in three consecutive cultures.

Results: We observed that *S. flexneri* in both 0.9% NaCl solution and distilled water survived the longest (87 and 83 days, respectively) when stored in the refrigerator. Similarly, *S. typhi* was found to be viable as long as 65 days in both 0.9% NaCl solution and distilled water, when stored in the refrigerator. No bacterial growth was determined in the cultures of chlorinated water samples stored at all four temperatures in the first 24 hours.

Conclusions: We concluded that *S. flexneri* generally survived longer than *S. typhi* in the different water media. Having a prolonged survival of *S. flexneri* in aqueous media would present more risk than *S. typhi* for the transmission of waterborne infections.

Key Words: Survival of bacteria, *Shigella flexneri*, *Salmonella typhi*, waterborne infections

Salmonella typhi ve *Shigella flexneri*' nin Çeşitli Su Örneklerinde ve Farklı Sıcaklıklardaki Yaşam Süreleri

Amaç: Bu çalışmada toplum sağlığı açısından önemli yeri olan *Salmonella typhi* ve *Shigella flexneri*'nin farklı sıcaklık derecelerindeki distile su, % 0.9NaCl içeren su ve klorlu su içinde canlı kalma sürelerinin değerlendirilmesi amaçlanmıştır.

Yöntem ve Gereç: Belirtilen su örneklerine yaklaşık 5×10^5 bakteri/ml olacak şekilde *S. typhi* ve *S. flexneri* karıştırıldı. Örnekler daha sonra 37 ºC, oda sıcaklığı, 4 ºC ve -20 ºC de bekletildi. Su örneklerinden ilk 24 saat sonrasında ve daha sonra günaşırı olacak şekilde üreme kontrol ekimleri yapılarak bu işleme 3 kez üst üste üreme saptanmayıncaya kadar devam edildi.

Bulgular: Çalışmamızda *S. flexneri*' nin en uzun süreyle, buzdolabında bekletilen %0.9 NaCl ve distile su içinde (sırasıyla 87 ve 83 gün) canlı kaldığı görüldü. Benzer şekilde *S. typhi*'nin de buzdolabında bekletilen % 0.9 NaCl ve distile su örnekleri içinde 65 gün boyunca canlı kaldığı saptandı. Dört farklı sıcaklıktaki klorlu su örneklerinden yapılan ekimlerin hiçbirisinde ilk 24 saatte üreme olmadı.

Sonuç: Çalışma sonunda *S. flexneri*'nin genel olarak su içeren ortamlarda *S. typhi*'den daha uzun süre hayatta kaldığı görülmüştür. Bu durum, su kaynaklı enfeksiyonlar açısından *S. flexneri*'nin *S. typhi*'ye göre daha büyük bir risk oluşturabileceğini göstermektedir.

Anahtar Sözcükler: Bakteri Yaşam Süresi, *Shigella flexneri*, *Salmonella typhi*, Su Kaynaklı Enfeksiyonlar

Received: July 02, 2007
Accepted: May 01, 2008

Correspondence
M. Hamidullah UYANIK
Department of Microbiology and Clinical Microbiology
Faculty of Medicine
Atatürk University
25240 Erzurum - TURKEY

mhuyanik@hotmail.com

Introduction

Salmonella and *Shigella* are the leading agents causing gastrointestinal infections, especially in developing countries. One of sources causing diseases with these bacteria in human beings is water, which is necessary to life and health. Waterborne diseases have been estimated to cause more than two million deaths and four billion cases of diarrhea annually (1). *Salmonella enterica* subspecies *enterica* serotype *typhi* (*S. typhi*) and *Shigella* species cause infection only in human beings. *S. typhi* colonizes only in humans and therefore, the disease can be acquired only through close contact with a person who has typhoid fever or is a chronic carrier. Most often, acquisition of organisms occurs by ingestion of food or water contaminated with human excreta (2). In 2003, the World Health Organization (WHO) estimated the annual occurrence of typhoid fever at 17 million cases, with approximately 600,000 deaths worldwide (3).

The infective dose for typhoid fever is between 10^3-10^7 organisms (4). Bacillary dysentery is the most communicable of the bacterial diarrheas (5). A small inoculums of *S. flexneri* (up to 100 organisms) is sufficient to cause infection (6). *Shigella* bacteria are transferred person-to-person by contaminated water and food. In developing countries, shigellosis is a common infection because of inadequate sewage disposal and lack of effectively treated water supplies. Most infections in developed countries are caused by *Shigella sonnei*, while in developing countries, *S. flexneri* predominates, as in our region (7).

Shigellosis is endemic throughout the world. There are approximately 164.7 million cases, of which 163.2 million are in developing countries and 1.5 million in industrialized countries. Each year 1.1 million people are estimated to die from *Shigella* infection and 580,000 cases of shigellosis are reported among travellers from industrialized countries. A total of 69% of all episodes and 61% of all deaths attributable to shigellosis involve children less than 5 years of age (8). In several investigations conducted, *S. flexneri* and *S. typhi* were reported to cause waterborne infections (9).

In our study, we aimed to determine the survival of *S. typhi* and *S. flexneri* in different water samples and at different temperatures.

Materials and Methods

Ten *S. typhi* and 10 *S. flexneri* strains isolated from samples of subjects with the suspected diagnosis of bacillary dysentery and enteric fever at Ataturk University Research Hospital, Microbiology Laboratory, were used as the test bacteria in the study. They were stored at -70°C until the study began. Before examination, bacteria were subcultured on MacConkey agar from stock cultures and pure strains were obtained. After biochemical and serological confirmation, bacteria on logarithmic phase were collected and comprised the survival study.

Three different aqueous media were used for performing of the survival studies: (a) 0.9% NaCl (physiological saline = SF), (b) 0.5 ppm chlorine tap water and (c) distilled water. Each medium was prepared with quantity of 100 ml in two series, one of which was used for *S. typhi* and the other for *S. flexneri*. The relevant bacteria were inoculated in each medium series with the final concentration of approximately $5x10^5$ bacteria/ml. From each of the water media containing *S. typhi*, 3 aliquots were collected into sterile 15 ml screw-capped tubes with the quantities of approximately 10 ml. Additionally 50 aliquots, each of approximately 1 ml, were collected into 2 ml sterile Eppendorf tubes. These proceedings were repeated for the other water media containing *S. flexneri*. Of the prepared water samples described above, one of each series of 10 ml tubes was kept at room temperature (18-24 °C) protecting from direct sunlight, the second at 37°C, and the third in the refrigerator (4-6 °C). The water samples collected in Eppendorf tubes were all kept in the deep freezer (-20 °C).

From the water samples kept in the stated environments, growth control cultures were done beginning in the first 24 hours and later every other day. For culturing from the samples kept in deep freezer, one tube was used each time in order to avoid thawing repeatedly. Culturing was ceased for samples demonstrating no growth in three consecutive cultures.

The process described above for one strain was repeated simultaneously with the other 9 *Salmonella* and *Shigella* strains. The survival (days) for the tested bacteria species in the relevant media and temperature was detected by taking the average of the values obtained from the 10 strains.

Results

Survival of the two bacteria species in three different aqueous media varied between <1 to 87 days according to the temperature at which they were stored. The results obtained from the study were as follows:

Survival time in 0.9% NaCl (physiological saline = SF): At 37°C, *S. typhi* remained viable for 5 days and *S. flexneri* for 45 days. At room temperature, *S. typhi* survived 29 days and *S. flexneri* 57 days. In the refrigerator, *S. typhi* remained viable for 65 days and *S. flexneri* for 87 days. In deep freezer; both *S. typhi* and *S. flexneri* remained viable for only 3 days.

Survival time in distilled water: At 37°C, *S. typhi* remained viable for 5 days and *S. flexneri* for 43 days. At room temperature, *S. typhi* survived 25 days and *S. flexneri* 57 days. In the refrigerator, *S. typhi* remained viable for 65 days and *S. flexneri* for 83 days. In the deep freezer, *S. typhi* died after 23 days, and *S. flexneri* after 27 days.

Survival time in chlorinated tap water: Both *S. typhi* and *S. flexneri* lost their viability within the first 24 hours in this media regardless of the temperature at which they were stored.

Both bacteria tested survived the longest at refrigerator temperature in 9% NaCl and in distilled water; however, *S. flexneri* remained viable longer in these conditions than *S. typhi*.

The average survivals of *S. typhi* and *S. flexneri* are shown in Table.

Discussion

Infections caused by *S. typhi* and *Shigella* species are a significant health problem in Turkey, as in several countries in the world. Water sources have an important role in infections caused by these bacteria. In this study, survival of *S. typhi* and *S. flexneri* at different temperatures and in different aqueous media was researched. According to our data, no growth was determined for both bacteria in the first passages of chlorinated water in the first 24 hours. This result highlights the importance of chlorination of tap water in the prevention of *S. typhi* and *S. flexneri* infections. In previous studies, Baumann and Ludwig (10) had determined that with the chlorination of water, 99.6-100% of *S. dysenteriae* had been inactivated in 10 minutes, while Korol et al. (11) determined that 99% of *S. typhi* had been inactivated in 6 minutes.

Based on the data we obtained, we can say that *S. typhi* survived for a shorter period than *S. flexneri* at all temperatures examined, and increase in temperature from 4°C to 37°C affected the survival of *S. typhi* negatively. Generally, with the decrease in temperature, increase in survival period of the bacteria is an expected result since the metabolism slows down at low temperature. However, in our study, survival periods of

Table. Survival of *S. typhi* and *S. flexneri* in three different aqueous media at different temperatures.

		S. typhi survival (days)	*S. flexneri* survival (days)
Incubator (37 °C)	0.9% NaCl	5	45
	Distilled water	5	43
	Chlorine tap water	<1	<1
Room temperature (18-24 °C)	0.9% NaCl	29	57
	Distilled water	25	57
	Chlorine tap water	<1	<1
Refrigerator (4-6 °C)	0.9% NaCl	65	87
	Distilled water	65	83
	Chlorine tap water	<1	<1
Deep freeze (-20 °C)	0.9% NaCl	3	3
	Distilled water	23	27
	Chlorine tap water	<1	<1

S. flexneri and *S. typhi* at -20°C were 3 days in SF, but in distilled water survival was 27 days for *S. flexneri* and 23 days for *S. typhi*. At 4°C, these periods were 65 and 87 days for the relevant bacteria, respectively. As registered from these data, both bacteria had survived for shorter periods at -20°C than at 4°C. The short survival of both bacteria at -20°C may be attributed to the formation of ice crystals (12).

Another data obtained from the study was that both bacteria had survived shorter periods in media containing SF than in distilled water at -20°C. This may be related to the increase in electrolyte imbalance in frozen media (13,14).

In previous studies, it had been determined that *Salmonella* species survived longer at +4°C than at 21 and 25°C (15,16). In the present study, we also determined that *S. typhi* survived longer at 4°C than at the other temperatures evaluated.

In one study conducted by Islam et al. (17), it was reported that *S. flexneri* survived longer at 4°C in 0.5% salt solution, but it survived longer at 25 and 37°C in distilled water. In our study, *S. flexneri* was found to survive longer in salt solution at 4°C and 37°C.

Consequently, based on our results, we have concluded that *S. flexneri* generally survived longer than *S. typhi* in all situations, and that distilled water and 0.9% NaCl solution are more convenient media for survival of the bacteria. Having a prolonged survival of *S. flexneri* in aqueous media would present more risk than of *S. typhi* for the transmission of waterborne diseases.

References

1. WHO, 2000. Water Supply and Sanitation Council, Global Water Supply and Sanitation Assessment 2000 Report. New York: UNICEF.
2. Miller SI, Pegues DA. *Salmonella* Species, including *Salmonella typhi*. In: Mandell GL, Bennet JE, Dolin R, editors. Principles and Practice of Infectious Diseases, 5th ed. New York: Churchill Livingstone Inc; 2000. pp. 2344-63.
3. WHO. Background document: The diagnosis, treatment and prevention of typhoid fever. Available: URL: http://www.who.int/vaccine_research/documents/en/typhoid_diagnosis.pdf
4. Janda JM, Abbott SL. The Enterobacteria, 2nd ed. New York: ASM Press; 2005. pp. 105-15.
5. DuPont HL, Levine MM, Hornick RB, Formal SB. Inoculum size in shigellosis and implications for expected mode of transmission. J Infect Dis 1989; 159: 1126-8.
6. Jennison AV, Verma NK. *Shigella flexneri* infection: pathogenesis and vaccine development. FEMS Microbiol Rev 2004; 28: 43-58.
7. Dupont HL. *Shigella* species (bacillary dysentery). In: Mandell GL, Bennet JE, Dolin R, editors. Principles and Practice of Infectious Diseases, 5th ed. New York: Churchill Livingstone Inc; 2000. p. 2363-9.
8. WHO. Available from: URL: http://www.who.int/vaccine_research/diseases/shigella/en/
9. World Health Organization (WHO). Water Recreation and Disease. Plausibility of Associated Infections: Acute Effects, Sequelae and Mortality by Kathy Pond. Published by IWA Publishing, London, UK. ISBN: 1843390663. Available from: URL: http://www.who.int/water_sanitation_health/bathing/recreadischap4.pdf
10. Baumann ER, Ludwig DD. Free Available Chlorine Residuals for Small Nonpublic Water Supplies. Am Water Works Assoc 1962; 54: 1379.
11. Korol S, Fortunato MS, Paz M, Sanahuja MC, Lazaro E, Santini P et al. Water disinfection: comparative activities of ozone and chlorine on a wide spectrum of bacteria. Rev Argent Microbiol 1995; 27: 175-83.
12. Heckly RJ. Preservation of microorganisms. Adv Appl Microbiol 1978; 24: 1-53.
13. Reimer LG, Carroll KC. Procedures for the storage of microorganisms. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, editors. Manual of Clinical Microbiology, 8th ed. Washington, DC: ASM Press; 2003. pp. 67-73.
14. Fonseca F, Marin M, Morris GJ. Stabilization of frozen *Lactobacillus delbrueckii subsp. bulgaricus* in glycerol suspensions: freezing kinetics and storage temperature effects. Appl Environ Microbiol 2006; 72: 6474-82.
15. Holliday SL, Beuchat LR. Viability of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in yellow fat spreads as affected by storage temperature. J Food Prot 2003; 66: 549-58.
16. Meckes MC, Johnson CH, Rice EW. Survival of *Salmonella* in waste egg wash water. J Food Prot 2003; 66: 233-6.
17. Islam MS, Rezwan FB, Khan SI. Survival of *Shigella flexneri* in artificial aquatic environment: effects of different physicochemical stress factors. J Diarrhoeal Dis Res 1996; 14: 37-40.