JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2016. Vol. **10**(1), p. 305-310

# Experimental Study of the Survival and Growth of *Pseudomonas aeruginosa* in Water Affected by Temperature, Storage Time and Type of Water

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(Received: 10 December 2015; accepted: 20 January 2016)

**In this inoculation study, a number of effective factors in the growth of *P.aeruginosa* in drinking water were investigated. This study was conducted with the inoculation of the bacterium at the level of $10^4$ /ml into drinking water and the factors investigated included: storage temperature of water (22 C° and 7C°), storage time of water (day zero, 3, 6, 12, 24, 48), type of mineral water (sterilized and non-sterilized) and tap water (sterilized and non-sterilized). The bacterium studied was *P.aeruginosa*. The main aim of this study was to reach a definite conclusion about the possibility of survival and growth of the bacterium in drinking water under the conditions mentioned. The different types of water were sampled and after preparing dilution, they were cultured in solid medium of BHI Agar and Cetrimid Agar and then they were incubated. In the end, storage temperature (22 C°) from the third day on, the sanitary condition of water (sterilized) from the 24th day on and the type of water source (mineral) on the 48th day had a positive effect on the growth of *P.aeruginosa*. Over all, temperature of water was the most effective factor in the growth and survival of *P.aeruginosa* as it had the highest growth at 22 C° water ($P<0.01$).**

**Keywords:** *Pseudomonas aeruginosa*, drinking water, growth, survival, temperature.

Recognizing and controlling pathogens and the way they are transmitted from the environment to human beings is of utmost importance. Water and food has the biggest role in transmitting diseases to human beings. A lot of infectious diseases and some non-communicable diseases could be transmitted through water and food. Some of these diseases can turn into a pandemic and cause a lot of deaths. Since health is the main component of sustainable development, and human beings health is highly dependent on healthy drinking water, the public health and welfare will be endangered without providing healthy drinking water. Enhancing the quality of drinking water and not using unsanitary water resources can play a key role in eliminating transmitted diseases. Removing pathogens from water is a high priority as they can cause pandemics and high rates of deaths. Some pathogens in water including *P.aeruginosa* called opportunistic pathogens do not become a health threat except in the elderlies and immunocompromised people[8, 9]. *P.aeruginosa* is a Gram-negative, rod-shaped, Catalase-positive and aerobic bacterium and grows

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best at 37 degrees C. The bacterium is commonly found in natural environments and is considered an opportunistic pathogen[4, 10]. In some studies, tap water has been reported to be a major source of *P.aeruginosa* nosocomial infection[3, 16, 17]. Mania, *et al*. (1990) isolated several species of gram negative bacteria including *P.aeruginosa* from mineral waters through heterotrophic plate count[7]. Also, Gonzalez and Tamgnini (1997) isolated *P.aeruginosa* in a study on bottled water[15]. In a 5-year inoculation study by Legnani, *et al*. (1999) on *P.aeruginosa*, the number of bacteria increased by 3 Log after 4-5 days (6). Bagheri Ardabilian, *et al*. (2013) isolated *P.aeruginosa* from the spa pools in Sarein[2]. Considering the facts mentioned above and the pathogenic importance of *P.aeruginosa*, this inoculation study aims to analyze the growth and survival of *P.aeruginosa* in drinking water affected by storage temperature, type of water, and storage time by inoculation of $1.5 \times 10^4$/ml of the bacterium into drinking water.

## MATERIALS AND METHODS

A multifactorial design was used to investigate the effects of temperature, inoculation dose, and type of water as a nutrient on the growth of *P.aeruginosa*. At first, a microbial experiment was conducted to investigate the existence of *P.aeruginosa* in the water samples. This experiment was conducted through 3-tube MPN1 method according to existing standards, both by Durham tube to analyze coliform bacteria and without Durham tube for general microbial analysis[14]. The result of the experiment showed no opacity in the analyzed tubes. The same experiments were conducted on inoculated water samples and the results were recorded. In order to prepare the first inoculation of the bacterium, the standard pure colony of *P.aeruginosa* with ATCC 27853 was taken to 10 ml BHI broth and was incubated at 30 C° and after 24 hours, 1ml of the first broth was taken to the second 10 ml broth and was incubated at 30 C°, and after 24 hours the bacterial count was conducted and full growth of the bacterium ($1.5 \times 10^9$ cfu) was achieved and then, standard dilution was conducted[14]. In order to achieve the inoculation dose of $1.5 \times 10^4$/ml, 0.1 CC from -2 dilution tube which contained $10^7$ cfu of the bacterium was carried to 100 CC of each of the water samples. In the end, the inoculation dose of the bacterium in each of the water samples was $1.5 \times 10^4$/ml. Then, in order to examine the growth and to draw the growth curve of the bacterium in samples at different times under sterilized and non-sterilized conditions, mineral and tap waters were sampled, and after preparing a dilution by surface plate count in BHI Agar, it was incubated at 30 C° for 24 hours [14]. After counting the colonies in plates, the results were recorded at different times (day zero, 3, 6, 12, 24, and 48). In order to achieve a higher precision, each of the stages and the combined factors were cultured in Cetrimid Agar as well as BHI Agar and the bacterial growth curve was drawn for the two media. There were 8 types of water samples in this study: sterilized bottled mineral water, non-sterilized bottled mineral water, sterilized tap water, non-sterilized tap water, each of which was stored at two storage temperatures of 7 and 22 C°. Considering the objective of the study, the culture was conducted at certain days (day zero, 3, 12, 24, 48), and the results were recorded.

## RESULTS

The results of bacterial counts in different dilutions of different types of water in BHI Agar and Cetrimide Agar were recorded. A specific dilution was chosen for each experiment and the logarithmic analysis of the data is presented in table 1.

This study was conducted by a $2^4$ factorial experiment with 16 treatments without repetition. The bacterial count results were changed into logarithm to base 10 before any statistical analysis. The methods REG, GLM, and ENTROPY and software SAS (version 9, 1) were used to analyze the data (SAS Institute Inc., 2002-2003). All the main and interactive effects were analyzed with the statistical p value set at $p < 0.05$.

**The survival and growth of bacteria in water**

On day zero (after the inoculation of the bacterium) there was no statistically significant difference between the effects of the factors on the growth of the bacterium and the arithmetic means of the logarithm of the bacterial count at the given time were 3.911 and 4.166 for mineral water

**Table 1.** The results of the logarithmic bacterial growth in a suitable dilution for each type of water

| Observation Type of water | | Day zero | Day 3 | Day 6 | Day 12 | Day 24 | Day 48 | Type of culture medium |
|---|---|---|---|---|---|---|---|---|
| Mineral 22° | sterilized | 4 | 6.471292 | 6.278754 | 6.257679 | 6.448706 | 6.41162 | BHI Agar |
| | Normal form | 4 | 6.326336 | 6.093422 | 5.812913 | 6.176091 | 6.462398 | BHI Agar |
| Mineral 7° | sterilized | 3.778151 | 4 | 3.778151 | Lab.Error | 5.758912 | 4.322219 | BHI Agar |
| | Normal form | 3.954243 | 4.633468 | 3.30103 | 3 | 3.30103 | 4.778151 | BHI Agar |
| Tap water 22° | sterilized | 4 | 6.808886 | 6.614897 | 6.363612 | 6.523746 | 6.518514 | BHI Agar |
| | Normal form | 4.845098 | 5.60206 | 5.30103 | 4.278754 | 3.60206 | 4.079181 | BHI Agar |
| Tap water 7° | sterilized | 4.544068 | 3.954243 | 3.477121 | 3 | 3.69897 | 4.845098 | BHI Agar |
| | Normal form | 3.845098 | 4 | 4.380211 | 4.69897 | 4.380211 | 3 | BHI Agar |
| Mineral 22° | sterilized | 4 | 4.357935 | 6.423246 | 6.303196 | 6.429752 | 6.346353 | Cetrimid Agar |
| | Normal form | 4 | 6.50515 | 5.139879 | 5.50515 | 6.217484 | 6.060698 | Cetrimid Agar |
| Mineral 7° | sterilized | 3.60206 | 3.477121 | 3.778151 | 3.30103 | 3.30103 | 4.146128 | Cetrimid Agar |
| | Normal form | 3.954243 | 3.845098 | 3 | 3.30103 | 3.778151 | 4.748188 | Cetrimid Agar |
| tap water 22° | sterilized | 4 | 6.634477 | 6.487138 | 6.334454 | 6.429752 | 6.307496 | Cetrimid Agar |
| | Normal form | 4.113943 | 5.544068 | 5.240549 | 4 | 3.69897 | 3.30103 | Cetrimid Agar |
| Tap water 7° | sterilized | 3.832509 | 3.623249 | 2.30103 | 3.146128 | 3.869232 | 4.725912 | Cetrimid Agar |
| | Normal form | 4.146128 | 3.845098 | 3.30103 | 3.60206 | 3.30103 | 3.30103 | Cetrimid Agar |

and tap water. The storage temperature of water had a big effect on the bacterial count three days after the beginning of the experiment and the other factors (in main and interactive effects) didn't have a noticeable effect on this feature. Considering the variance analysis results for this feature, storage temperature of 22 C° with the mean of 6.031 meaningfully (P<0.01) increased the number of the bacteria compared to temperature storage of 7 C° with the mean of 3.922. Six days after the beginning of the experiment the main effective factor in the growth of the bacteria was storage temperature too. Also, analyzing the bacterial count on day twelve showed the storage temperature of 22 C° compared to that of 7 C° (with corresponding means of 5.607 and 3.436, respectively) had a statistically meaningful effect on the growth of the bacteria (P<0.01) . On day twenty four also the main effective factor was storage temperature followed by the sanitary condition of water. In this regard, storage temperature of 22 C° compared to that of 7 C° (with corresponding means of 5.691 and 3.924, P<0.01, P<0.01) and using sterilized water compared to non-sterilized tap water (with corresponding means of 5.308 and 4.307, P<0.01, P<0.05) were more effective in the growth of the bacteria. On day forty eight, storage temperature of 22 C° (compared to that of 7 C°, with corresponding means of 5.686 and 4.233), using bottled mineral water (compared to tap water, with corresponding means of 5.409 and 4.510) and using sterilized water (compared to non-sterilized water with corresponding means of 5.453 and 4.466) significantly increased the multiplication of the colonies.

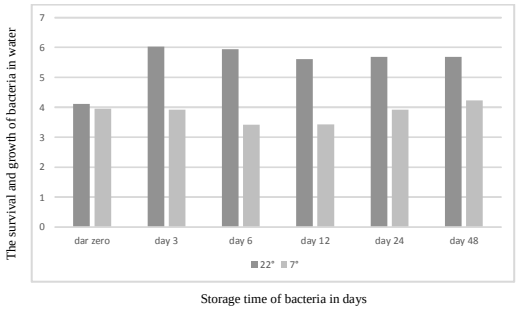


**Diagram 1.** The effect of temperature of waters at different times of the experiment

## DISCUSSION

In this study the effect of storage time, temperature of water, sanitary condition of water on the growth of *P.aeruginosa* in drinking water (mineral and tap water) was investigated. The results showed that *P.aeruginosa* grows well in water and among all factors studied here, temperature of water proved the most effective on the growth and survival of the bacterium. The most bacterial count was in 22 C° water (P<0.01). However, due to being cold loving, as more time passes, the bacterium may grow as much as it does in 22 C°. Also, the bacterium continues to survive in water after 48 days. On day zero (after inoculation) no factor was effective on the growth of the bacterium, but after 3 days, temperature of water had a great effect on the growth of the bacterium. The effect of temperature continues to exist from day 3 until day 48 and the effect of 22 C° water was more than that of 7 C° water and this is because generation time of the bacterium is shorter at higher temperature. From day 12 on, the interactive effect of temperature (22 C°) and the sanitary condition of water was significantly meaningful and the sanitary condition of water

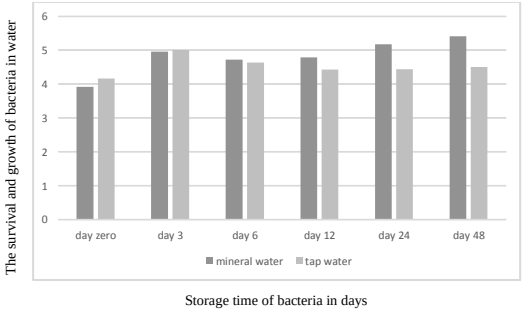


**Diagram 2.** The effect of type of water at different times of the experiment

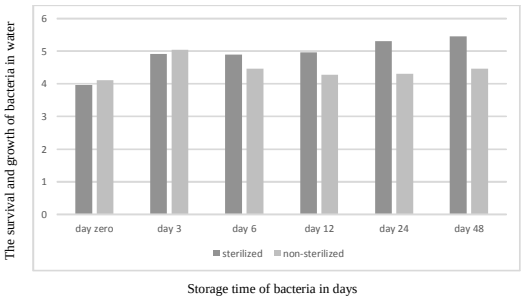


**Diagram 3.** The effect of sanitary condition of waters at different times of the experiment

(sterilized and non-sterilized) from day 12 until day 48 had a positive effect on the growth of the bacterium i.e. the growth of the bacterium was more in sterilized than in non-sterilized water ($P<0.05$). on day 24, the most effective factor was temperature (22 C°) followed by sanitary condition of water i.e. the growth of the bacterium was more in sterilized than in non-sterilized water and this could be due to the interruption of normal flora of water by sterilization and the lack of microbial competition for the growth of *P.aeruginosa*. 48 days after the storage of waters, type of water also was an effective factor i.e. the growth of the bacterium in mineral water was more than that in tap water ($P<0.01$) and this could be due to the abundance of mineral contents required for the growth of the bacterium in mineral water compared to those in tap water. Water temperature (22C°) and sanitary condition of water (sterilized) were effective and caused an increase in the growth of the bacterium. Generally, the effect of temperature was the most effective factor throughout the study.

Manaia *et al*. (2008) studied 15 brands of noncarbonated bottled mineral water and isolated *P.aeruginosa*. The results of their study, too, showed the positive effect of type of water (mineral) on the growth of *P.aeruginosa*[7]. Blance *et al*. (2004) mentioned faucets of intensive care units of hospitals as an endemic source of *P.aeruginosa*. Studying persistence of the bacterium was part of this study and the bacterium was shown to exist in water after 48 days[3]. Legnani *et al*. (1999) in a 5-year study on two-tailed growth curve of *P.aeruginosa* inoculated at the density of $10^2$ cfu/ml in samples of natural mineral water found that the number of bacteria increased by 3 Log after 4-5 days. The results of this study too, showed the positive effects of type of water (bottled mineral water) and storage time on the growth of *P.aeruginosa*[6]. Bagheri *et al*. (2013) isolated *P.aeruginosa* from the spa pools in Sarein. The results of this study also showed the effect of type of water (mineral) and storage time on the growth of *P.aeruginosa*[2].Baghal Asghari *et al*. (2013) by analyzing Pseudomonas Infections found in hospitals concluded that detecting the source of pollution is a high priority in preventing such infections in hospitals. This study also found type of water as a positive factor on the growth of *P.aeruginosa*[1].

Analyzing the survival and growth of *P.aeruginosa* in bottled mineral waters (sterilized and non-sterilized ) and tap waters (sterilized and non-sterilized ) at temperatures of 22 and 7 C° in storage time of 48 days after the inoculation shows that the bacterium has a noticeable growth in water and storage temperature (22 C°) ($P<0.01$) and sanitary condition of water (sterilized) ($P<0.05$) and type of water (mineral) ($P<0.01$) have a positive effect on the growth of *P.aeruginosa*. All in all, water temperature was the most effective factor in the growth and survival of the bacterium i.e. the bacterium had the greatest growth at 22 C° ($P<0.01$). Considering the survival of the bacterium 48 days after the inoculation, it is suggested that its growth be investigated after 96 days after inoculation at 37 C° which is the desired temperature for the growth of the bacterium.

## REFERENCES


1. Baghal Asghari, F. Nik Ayin, M. The importance of Biofilms of water systems in infection´s spread of Pseudomonas aeroginosa in hospital 2013.
2. Bagheri Ardebilian, P. Sadeghi, H. Fazlzadeh davil, M. Rostami, R. Poureshgh Y. Pseudomonas aeroginosa in Public Hot Spring Pools of Sareyn country 2013.
3. Blanc, D.S. Nahimana, I. Petignat,C. Wenger, A. Bille,J. Francio,P., Faucets as a reservoir of endemic Pseudomonas aeruginosa colonization/ infections in intensive care units, 2004; (10). pp 1964-1968.
4. Jawetz, Melnick and Adelberg's Medical microbiology, Twenty. Fifth Edition– LANGE Basic Science.
5. Jay, J.M., Modern Food microbiology, Fourth Edition, chapman and Hall, Newyurk, 1992; pp: 4695-469.
6. Legnani, P. Leoni,E. Rapuano,S. Turin,D. Valenti, C., Survival and growth of Pseudomonas aeroginosa in natural mineral water: a 5- year study, *International Journal of Food Microbiology*, **53**(2-3): Pages 153-158.
7. Manaia, Célia. Nunes,Olga C. Morais,Paula V. and Dacosta,M. Heterotrophic Plate counts and the isolation of bacteria From mineral waters on selective and enrichment media, 1990; **69**: 871-876.
8. Nabizadeh, R. Faezi Razi, D. Drinking water quality guidelines 1996.
9. Nami Fard, Zahra. Water borne diseases 2010.

10. Noroozi, J. Pathogenic bacteria 2008.
11. Razavilar,V. Pathogenic Microorganisms in Foods and Epidemiology Food Poisoning 2002.
12. Razavilar. V. and C. Lenigeorgis. Interactive effect of trmperature,atmosphere and storage time on the Probability of colony formation on blood agar by four listeria Species. *J. Food. Prot,* 1992; **55**: 88-92.
13. Razavilar, V. Hazard analysis critical control point (HACCP) and Modeling In: Proc., Food Safety sym posium. Univ Of medical Sciences of Tehran,Iran 1997.
14. Speck, marvin L. Compendium of methods for the microbiological examination of foods. Prepared by the APHA intersociety Agency committee on microbiological methods for foods 1984.
15. Tamagnin, L.M and Gonzalez, r.D. Bacteriological Stability and growth kinetics of Pseudomonas aeruginosa in bottled water, Article First published online: 25 NOV 2003, *Society for Applied Bacteriology,* 1997; **83**: 91-94.
16. Trautmann, Matthias. Lepper, Philipp M. Haller, Mathias. Ecology of Pseudomonas aeruginosa in the intensive care unit and the evolving role of water outlets as a reservoir of the organism, 2005; **33**(5): Supplement, Pages S41-S49.
17. Trautmann, Matthias. Michalsky, Thomas. Wiedeck, Heidemarie. Radosavljevic, Vladan. Ruhnke, Markus. Tap Water Colonization With Psmdomonas aeruginosa in a Surgical Intensive Care Unit (ICU) and Relation to Psmdomonas Infections of ICU Patients, 2001; : 49-52.
18. Varnan, A.H., E vans, M.G.(2005). Food borne Pathogens, an illustrated text. 3th edition, manson publications.
19. Walls,l.and scotl, V.N. Use of predictive microbiology in microbial food safety risk assessment. *Int. J. Food Microbial,* 1997; **36** (2-3): 97-102.