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RESEARCH ARTICLE

Long Term Survival of *Pseudomonas aeruginosa* in Distilled Water and in Normal Saline

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Abstract

Pseudomonas aeruginosa is an important opportunistic pathogen primarily causing nosocomial infections in immunocompromised patients and is responsible for high mortality rates in burn centers, it can be isolated from hospitals, from the water in intensive care units, as well as sinks, basins, drains, showers, toilets and bathtubs, leading to transmission of *P. aeruginosa* infections. To determine the physiological adaptations which allow *P.aeruginosa* to survive in aqueous environments, we performed this experiment by transfer the bacterial cultures from Luria broth to distilled water and normal saline and incubate for 6 months and 12 months respectively. *P. aeruginosa* was able to survive in distilled water for at least 180 days(6 months) and in normal saline for more than 360 days(12 months). We also examined the ability of this cells to pyocyanin production and antibiotic resistance, and the results showed that *P. aeruginosa* cells were able to produce pyocyanin and resist to cefotaxime , augmentin , ceftazidime cefepime and sensitive to imipenem , norfloxacin and gentamicin. The results of this study revealed that *P. aeruginosa* bacteria can be preserved in distilled water or normal saline for several months, and able to pyocyanin production as well as antibiotic resistance. It is important to understand how this bacteria survives in water. Understanding the mechanisms of survival in water may contribute to novel solutions for the prevention of *P. aeruginosa* infection and transmission.

Keywords: *Persistance, Preservation, Saline, Water.*

Introduction

The medical community is fighting a war against *Pseudomonas aeruginosa*, as mortality rates associated with the bacteria remain consistently high, *P. aeruginosa* can thrive and survive in a variety of temperature and infrequent nutrition [1]. *P. aeruginosa* has been shown to survive in water, significantly longer than *Escherichia coli* and *Staphylococcus aureus* [2].

The ability of the bacteria to persist at length in water indicates that water may be an environmental reservoir for *P. aeruginosa*, the microbiome of pristine groundwater is dominated by the *Pseudomonas* genus, which was shown to represent 10% of all species [3].Numerous studies have demonstrated that *P. aeruginosa* can be isolated from hospitals, from the water in intensive care units, as well as sinks, basins, drains, showers, toilets and bathtubs, leading to transmission of *P. aeruginosa* infections [4]. It is therefore important to understand how this bacteria is able to survive in water, to better understand the transmission and possibly improve infection control policies [3]. A study was done by Wuthiekanun *et al* [5]. Demonstrated the ability of *Burkholderia pseudomallei* to survive in double distilled water for 3 years.

Water supplies in Australia have been connected to meliododosis cases, Currie *et al* [6].Illustrating the threat that long term survival in water may pose to water supplies. The molecular mechanisms which allow *B. pseudomallei* to survive in water are poorly understood [7]. This study is aimed to determine the physiological adaptations which allow *P.aeruginosa* to survive in aqueous environments.

Materials and Methods

Bacterial Isolates used in this Study

Pseudomonas aeruginosa isolates from burn infections were grown in Luria broth and incubated at 37°C for 24 hrs. Logarithmic phase cultures were obtained by sub-culturing overnight cultures into Luria broth and growing these to an optical density (OD600) of 0.5 before being inoculated into distilled water and normal saline for experimental procedures and incubate for 6 months and 12 months respectively. Samples were removed from DW and normal saline at a number of time points to determine the viable cells.

Antibiotic Susceptibility Testing

The susceptibility of isolates to different antibiotics was tested using the Kirby-Bauer disk diffusion method following the Clinical and Laboratory Standards Institute guidelines [8], using antibacterial agents included: gentamicin (GM), Norfloxacin (NOR), ceftazidime (CAZ), cefotaxime (CTX),Cefepime (FEP), augmentin (Aug), carbenicillin (PY), and imipenem (IMP), on Mueller- Hinton agar plate (Himedia, India) using overnight culture at McFarland standard 0.5 followed by incubation at 35°C for eighteen hours.

Phenotypic Detection of Pyocyanin Production

Pyocyanin production was detected on King's B medium [9] and Mueller-Hinton agar media by streaking the overnight culture followed by incubation at 30°C for twenty-four hours.

Results and Discussion

Pseudomonas aeruginosa is a Gram-negative bacterium that is ubiquitous in the environment and appreciated for its ability to cause disease in plants, insects, animals, and humans. Kramer *et al* [10].Demonstrated that *P. aeruginosa* may survive for months on hospital surfaces. *P. aeruginosa* is an archetypal biofilm forming organism, which is a conserved strategy used for long-term survival in nature and during infections [3].

After storage in sterile distilled water for 6 months and in normal saline for 12 months , *P.aeruginosa* remained viable and could be recovered by plating the preserved culture on nutrient agar media, BHIA and Muller Hinton agar (Table 1). The recovered bacteria retained the ability to pyocyanin production and resist to cefotaxime, augmentin, ceftazidime cefepime and sensitive to imipenem, norfloxacin and gentamicin (Table 2).

Table 1: Population changes of *Pseudomonas aeruginosa* bacteria from cultures preserved in sterile distilled water and normal saline for different number of months

Time	The number of Viable cells in Distilled water(cell/ml)	The number of Viable cells in Normal saline (cell/ml)
After 1 months	2.5 X 10^4	2.9 X 10^4
After 2 months	1.9 X 10^4	2.5 X 10^4
After 3 months	1.6 X 10^4	1.9 X 10^4
After 4 months	1.4 X 10^4	1.6 X 10^4
After 5 months	1.4 X 10^4	1.6 X 10^4
After 6 months	-	1.6 X 10^4
After 7 months	-	1.6 X 10^4

: Not tested

By comparison, the viable cells of *P. aeruginosa* continued to decline after storage in distilled water for at least 180 days(6 months), but at a relatively slower rate. However, when stored in normal saline, the declines in the populations occurred mainly after 360 days(12 months) of storage. bacteria appear to survive better in normal saline than in water.

Table 2: Antimicrobial susceptibility test results of *P. aeruginosa* isolates

Antimicrobial agents	Antibiotic susceptibility of recovered bacteria after 12 months
gentamicin	S
norfloxacin	S
ceftazidime	R
cefotaxime	R
cefepime	R
augmentin	R

carbenicillin	R
imipenem	S

The ability of the organism to persist at length in water indicates that water may be an environmental reservoir for *P. aeruginosa*. Liao and Shollenberger [11] recorded only a small proportion of bacteria (1% or less) survived after storage in water or PBS for 30 weeks. *Agrobacterium tumefaciens* can be maintained in pure water for several years [12]. Food-borne pathogen, *Salmonella typhi*, have been shown to survive in tap water for up to 7 days [13] and *E. coli* strains in ground water for at least 132 days [14].

Most Gram-positive bacteria, such as Enterococcus spp. (including VRE), *Staphylococcus aureus* (including MRSA), or *Streptococcus pyogenes*, survive for months on dry surfaces. Many Gram negative bacteria, such as Acinetobacter spp., *Escherichia coli*, *Pseudomonas aeruginosa*, Klebsiella spp., *Serratia marcescens*, or Shigella spp., can also survive for months. A few others, such as *Bordetella pertussis*, *Proteus vulgaris*, *Haemophilus influenzae*, or *Vibrio cholerae*, however, persist only for days [10].

During outbreaks, the environment may play a significant role for transmission of nosocomial pathogens, as suggested by observational evidence. This has been described for various types of bacteria, such as *Acinetobacter baumannii* [15], *Clostridium difficile* [16] *Pseudomonas aeruginosa* [17] and VRE [18].

Long-term survival of food-borne pathogens in water underlines the importance of water as a potential vehicle for transmitting the diseases [11].The data presented here affirm the dependability of using distilled water or normal saline for preservation of *P.aeruginosa*.This method is a simple and economical means for preservation of bacterial cultures, which is especially useful for laboratories not equipped with the lyophilizer or ultra-low freezer. There are numerous experimental systems used to study Non growing bacteria, which include persister cells, starved cells in stationary phase, or the viable- but nonculturable state [19]. All of these non-growing states can be considered a form of dormant bacterial cells.

Non-growing persister cells are present in laboratory grown planktonic and biofilm cultures, and contribute to multidrug antibiotic tolerance and chronic infections (20). Persister cells are not utilizing nutrients, producing proteins, synthesizing any replication machinery, and therefore not multiplying [3].Inspite of the simplicity, preservation of bacteria in pure water has not been widely adopted in most of the microbiological laboratories. Furthermore, only a small number of bacteria, mainly phytopathogens have been tested in the past. Similar to our results, Jubair *et al* [21].

Reported that *Vibrio cholerae* has been shown to shift to a persister phenotype in water. When *V. cholerae* was introduced into filter sterilized lake water the cells displayed characteristics of persister cells and were culturable for >700 days. Interestingly, these authors also observed that the cells became smaller and formed aggregates over time in water.

The approach of *P. aeruginosa* incubated in water may be useful to study antibiotic tolerance and the mechanisms of dormancy and survival in nutrient limiting conditions. It is therefore important to understand how this organism is able to survive in water, to better understand the transmission and possibly improve infection control policies.

References

1. Jenny M, Kingsbury J Properties (2018) Prevention: A Review of Pseudomonas aeruginosa. J. Biol. Med. Res., 2(3):18
2. Moore RA, Tuanyok A, Woods DE (2008) Survival of Burkholderia pseudomallei in water. BMC Res Notes. 1;11. https://doi.org/10.1186/1756-0500-1-11.
3. Lewenza S, Abboud J, Poon K, Kobryn M, Humplik I, Bell JR, et al (2018) Pseudomonas aeruginosa displays a

dormancy phenotype during long-term survival in water. PLoS ONE 13: 9.

4. Aumeran C, Paillard C, Robin F, Kanold J, Baud O, Bonnet R (2007) Pseudomonas aeruginosa and Pseudomonas putida outbreak associated with contaminated water outlets in an oncohaematology paediatric unit. J Hosp Infect. 65(1);47±53. https://doi.org/10.1016/j.jhin.2006.08.009.
5. Wuthiekanun V, Smith MD, White NJ (1995) Survival of Burkholderia pseudomallei in the absence of nutrients. Trans. R. Soc. Trop. Med., 89(5):491.
6. Currie BJ, Mayo M, Anstey NM, Donohoe P, Haase A, Kemp DJ(2001) A cluster of melioidosis cases from an endemic region is clonal and is linked to the water supply using molecular typing of Burkholderia pseudomallei isolates. Am J. Trop. Med. Hyg., 65(3):177-9. e0198384. https://doi.org/10.1371/journal.
7. Richard A Moore , Apichai Tuanyok, Donald E Woods (2008) Survival of Burkholderia pseudomallei in Water., BMC Research Notes .,1:11 doi:10.1186/1756-0500-1-11
8. CLSI (2011) Performance standard for antimicrobial susceptibility testing; TwentyFirst informational supplement, 31(1): 100-S21.
9. King EO, Ward MK, Raney DE (1954) Two simple media for the demonstratoin of pyocyanin and fluorescein. J. Lab. Clin Med., 44:301-307.
10. Kramer A, Schwebke I, Kampf G (2006) How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infectious Dis., 6: 130. https://doi.org/10.1186/1471-2334-6-130 PMID.
11. Liao CH, Shollenberger LM (2003) Survivability and long-term preservation of bacteria in water and in phosphate-buffered saline, Letters in Applied Microbiology, 37: 45-50.
12. Iacobellis NS, DeVay JE (1986) Long-term storage of plant pathogenic bacteria in sterile distilled water. Applied and Environmental Microbiology, 52: 388-389.
13. Mitscherlich E, Marth EH (1984) Microbial survival in the environment: bacteria and rickettsia important in human and animal health. 802. Berlin: Springer-Verlag.
14. Banning N, Toze S, Mee BJ (2002) Escherichia coli survival in groundwater and effluent measured using a combination of propidium iodide and the green fluorescent protein. Journal of Applied Microbiology, 93: 69-76.
15. Fierobe L, Lucet JC, Decre D, Muller-Serieys C, Deleuze A, Joly-Guillou ML, Mantz J, Desmonts JM (2001) An outbreak of imipenem-resistant Acinetobacter baumannii in critically ill surgical patients. Infection Control and Hospital Epidemiology, 22:35-40.
16. Hanna H, Raad I, Gonzalez V, Umphrey J, Tarrand J, Neumann J, Champlin R (2000) Control of nosocomial Clostridium difficile transmission in bone marrow transplant patients. Infection Control and Hospital Epidemiology, 21**:**226-228.
17. Lemmen SW, Hfner H, Zolldann D, Stanzel S, Lütticken R (2004) Distribution of multi-resistant Gram-negative versus Gram-positive bacteria in the hospital inanimate environment. Journal of Hospital Infection, 56:191-197.
18. Falk PS, Winnike J, Woodmansee C, Desai M, Mayhall CG (2000) Outbreak of vancomycin-resistant enterococci in a burn unit. Infection Control and Hospital Epidemiology, 21: 575-582.
19. Bergkessel M, Basta DW, Newman DK. (2016) The physiology of growth arrest: uniting molecular and environmental microbiology. Nat. Rev. Microbiol., 14(9): 549-562. https://doi.org/10.1038/nrmicro.2016.107.
20. Lewis K (2007) Persister cells, dormancy and infectious disease. Nat Rev Microbiol. 5(1);48-56. https:// doi.org/10.1038/nrmicro1557 .
21. Jubair M, Morris JG Jr, Ali A (2012) Survival of Vibrio cholerae in Nutrient-Poor Environments Is Associated with a Novel "Persister" Phenotype. PLoS ONE 7(9): e45187. doi:10.1371/journal.pone.0045187.

