Faculty of Resource Science and Technology

Occurrence of *Pseudomonas aeruginosa* in Drinking Water from Water Vending Machine (WVM)

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

The rise in popularity of water vending machine (WVM) due to urban modernization has given a rise to concerns regarding the quality and safety of the drinking water. Several data have shown the potential risk WVM could harbor. However, there is a lack of such data on its risk in Sarawak. Some studies have found that there is a potential risk of WVM being contaminated with the opportunistic pathogen *Pseudomonas aeruginosa*. In this research, the level of *P. aeruginosa* prevalence in WVM is studied. Over the course of 2 months from January 2021 to February 2021, sampling was done once a month from WVM located in Kota Samarahan and Kuching. A total of one hundred drinking water samples (n=100) were taken from Kota Samarahan (n=50) and Kuching (n=50). The physical condition, surrounding location, date of maintenance and whether the WVM had a licensed were observed. The drinking water were analyzed for pH, total dissolved solids (TDS) and temperature. Enumeration and isolation of *P. aeruginosa* was done using single dilution method for most probable number (MPN). Identification was done with polymerase chain reaction (PCR) targeting the ETA gene, Antibiotic susceptibility test (AST) done according to the M45 guideline. Biofilm forming capacity of *P. aeruginosa* was studied in vitro. The results concluded that 26% of WVM from Kota Samarahan and 36% from Kuching were positive for the presence of ETA positive *P. aeruginosa* and it was observed that many of the WVM were not in acceptable condition including the absence of dispensing area cover, low hygiene condition, unsanitary or unsuitable locations and a lack or absence of maintenance done to the WVM. The AST conducted showed that *P. aeruginosa* was most susceptible to Meropenem (64%) and it was most resistant to ciprofloxacin (28%). The biofilm forming capacities were mostly observed to be moderate strength with 5 moderate strength and only 1 non-adhering based on *P. aeruginosa* biofilm-forming capacity in vitro. It is recommended that a stricter monitoring of WVM is needed to ensure the safety of the public.

Keywords: *Pseudomonas aeruginosa*, water vending machine (WVM), most probable number (MPN), ETA gene, biofilm

Kejadian Pseudomonas aeruginosa dalam Air Minuman Daripada Mesin Jual Air (MJA)

ABSTRAK

Pemodenan bandar membawa kepada kenaikan populariti water vending machine (WVM). Ini meningkatkan soalan tentang kualiti dan keselamatan air minuman MJA.Terdapat beberapa data mengenai potensi risiko daripada MJA. Bagaimanapun, terdapat kekurangan data mengenai hal ini di Sarawak. Terdapat beberapa penyelidikan yang menunjukkan MJA ada potensi untuk dicemari oleh bakteria Pseudomonas aeruginosa, *Penyelidikan ini dijalankan untuk mengetahui tahap kelaziman* P. aeruginosa *dalam MJA. Sampel diambil dalam tempoh 2 bulan, dari Januari 2021 hingga Februari 2021 sekali sebulan. Sebanyak 50 sample minuman air (n=25) daripada Kota Samarahan (n=25) dan Kuching (n=25). Keadaan fisikal, keaadaan kawasan sekeliling, tarikh servis dan kehadiran lesen MJA telah diperhatikan. Analisis pH, jumlah pepejal terlarut, dan suhu air minuman telah dilakukan. Enumerasi dan isolasi* P. aeruginosa *telah dilakukan menguna teknik dilusi tunggal bilangan paling berkemungkinan.* P. aeruginosa *telah diidentifikasi menggunakan teknik tindakbalas rantai polymerase menyasar gen ETA. Ujian ketahanan terhadap antibiotik dijalankan menggunakan garis panduan M45. Kebolehan* P. aeruginosa *untuk membentuk biofilem dikaji secara vitro. Hasil daripada penyelidikan ini menunjukkan 26% MJA daripada Kota Samarahan dan 36% daripada Kuching adalah positif kepada kehadiran ETA positif* P. aeruginosa *dan banyak MJA dijumpai dalam keaadan yang tidak menyenangkan seperti ketiadaan penutup bahagian pendispensan air, tahap kebersihan rendah, kawasan sekitar yg tidak bersih ataupun tidak sesuai dan kekurangan atau ketiadaan penyelenggaraan dilakukan terhadap MJA . Ketahanan terhadap antibiotik menunjukan tahap kelemahan paling tertinggi kepada Meropenem (64%) dan tahap* *ketahanan paling tinggi kepada ciprofloxacin (28%). Kapasiti* P. aeruginosa *untuk membentuk biofilem adalah sederhana dengan 5 mununjukkan kadar ketahanan sederhana dan hanya 1 menunjukkan ketiadaan ketahanan berdasarkan kapasiti* P. aeruginosa *untuk membentuk biofilem dalam vitro. Adalah disyorkan utuk meningkatkan pemantauan yang lebih tegas bagi memastikan keselamatan orang awam.*

Kata kunci: Pseudomonas aeruginosa, *water vending machine (WVM), bilangan paling berkemumkinan, gen ETA, biofilem*

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LIST OF ABBREVIATIONS

AGE	Agarose gel electrophoresis
AST	Antibiotic susceptibility test
ATTC	American type culture collection
CF	Cystic fibrosis
CFU	Colony forming unit
ddH_2O	Deionized distilled water
DNA	Deoxyribonucleic acid
EOW	Electrochemically oxidized water
ERW	Electrochemically reduced water
ETA	Exotoxin A
GAC	Granular activated carbon
HAP	Hospital-acquired pneumonia
HCAP	Healthcare-associated pneumonia
HPC	Heterotrophic plate count
LB	Luria-Bertani
LPS	Lipopolysaccharide
MHA	Mueller Hinton agar
MIC	Minimum Inhibitory Concentration
MPN	Most probable number

MOH	Malaysian Ministry of Health
OD_c	Cutoff value
OMV	Outer membrane vesicle
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
ppm	Part per million
QMRA	Quantitative microbial risk assessment
RO	Reverse osmosis
ROC	Reverse osmosis concentrate
SFA	Singapore Food Agency
TDS	Total dissolved solids
T3SS	Type III secretion system
T6SS	Type VI secretion system
TOC	Total organic carbon
TSB	Tryptic soy broth
TSS	Total suspended solids
UV	Ultraviolet
VAP	Ventilator-associated pneumonia
WHO	World Health Organization
WVM	Water Vending Machine

CHAPTER 1

INTRODUCTION

1.1 Study Background

In this current age of modernisation and public infrastructure a need for accessibility to the basic human necessities such as water is a must. This has caused the increase in popularity of machines such as water vending machine (WVM). Water vending machine (WVM) is an automated device which dispenses out clean filtered drinking water when the appropriate amount of money is inserted. Such machines provided a convenient access to potable drinking water at a cheap price for as low as 10 cents per litre. They were usually located in convenient places such as grocery stores, markets and housing areas. These machines provided an alternative to boiling tap water which takes up time and have an unfavourable taste due to the presence of toxins from leaking pipes and corrosion (Fauzi et al., 2020). According to Doria (2006), consumers often opt for alternative sources to drinking water other than tap due to 2 main reason, taste and health concerns as well as other factors. Hence, with factors which included convenience, safety and price, consumers would usually opt for water from WVM. However, filtered water from WVM could also became unsafe to consume due to risk factor such as improper maintenance, usage and low hygiene. This would introduce unwanted pathogens and bacteria into the filtration system, compromising the cleanliness and the safety level of the water dispensed.

There had been a few cases reported on the contamination of WVM in Malaysia. On 19 August 2014, The Star Online reported that almost all the drinking water sample taken from

WVM around the Klang Valley area were contaminated with harmful bacteria and that many of the samples did not have any traces of free chlorine or chloride which is needed for disinfection. (Aruna & Cameons, 2014). Another case on 2 September 2015 where 630 WVM and 786 water sample were tested in which 22 machines were confiscated where 4 had low hygiene levels and 10 were positive for presence of bacteria (Bernama, 2015). This was the result of poor maintenance and hygiene of the WVM. One particular case involved the detection of the bacterium *Pseudomonas aeruginosa* in a Malaysian brand of bottled drinking water done by the Singapore Food Agency (SFA) on 29 June 2019 (Bernama, 2019).

Pseudomonas aeruginosa is a Gram-negative aerobic bacterium with a rod shape. It is a non-spore forming bacterium and an oxidase-positive and a lactose non-fermenter. It is also ubiquitous meaning it could be found thriving anywhere in the natural environment such as soil, water, vegetation and even the human body. Part of what make *P. aeruginosa* significant in the scientific research community was the fact that it is an opportunistic pathogen that had caused a wide variety of infections especially amongst the immunocompromised individual such as cystic fibrosis (CF) patients and patients with severe wounds or burns. It took very low maintenance for *P. aeruginosa* to had flourished successfully in its environment. It survived in hospital setting and colonised the medical devices and also the water system. This gave it a higher chance for the bacteria to come into contact with immunocompromised individuals. Its multi-drug resistance was what made it hard to be treated in patients and to be eradicated from the water system. An array of virulence factor such as toxins and surface molecules also facilitated in its persistence and adaptability. There was also the fact that this bacterium could form biofilms providing an added layer of protection against antibiotics and the immune system.

Biofilms would form inside the water system and would be difficult to remove once established because chemical treatment or physical removal is needed.

1.2 Problem Statement

As the availability and usage of WVM increased due to factors such as convenience and low prices, there was an increased in concern of the cleanliness and the quality of the drinking water. This was due to the fact that several cases had reported that these machines were prone to bacterial contamination due to the lack of regular and proper maintenance and improper usage by the consumers. Because of the poor maintenance provided to these machines there was a concern for potential contamination of the opportunistic pathogen *P. aeruginosa*.

Under normal circumstances, *P. aeruginosa* would not cause severe harm or infections in healthy individuals. Most cases of *P. aeruginosa* infections were reported in hospital settings involving immunocompromised individuals but there had been cases linked to contaminated drinking water. Although rare and these cases were usually linked to already vulnerable individuals due to pre-existing medical conditions. It is important that precautious measures are taken.

Despite the risks that *P. aeruginosa* possesses due to its opportunistic nature, there is a lack of data on its occurrence in drinking water specifically from WVM. There were some researches pertaining to this issue conducted within Malaysia. However, there was a lack of it being done in Sarawak. Extensive research on the level of contamination of *P. aeruginosa* in drinking water from WVM was needed. These knowledges were needed to build effective strategies to prevent the presence of *P. aeruginosa* and ensured the safety of drinking water available to the public. Based on previous studies done on this topic, it was hypothesised that

WVM that adhere to the rules and regulations and strict hygiene practices, will exhibit lower level of *P. aeruginosa* contamination compared to those that does not adhere to the standard regulations and proper hygiene practices.

1.3 Objectives

This research aimed to fill in the knowledge gap about the extent of the contamination of *P. aeruginosa.* This was achieved by using the following objectives:

i. Evaluation of the drinking water samples from water vending machine (WVM) around Kota Samarahan and Kuching using physical and chemical parameters
ii. Profiling of *P. aeruginosa* from water vending machine (WVM)
iii. Investigation of the resistance profile of *P. aeruginosa* through antibiotic susceptibility test from water vending machine (WVM)
iv. Analysation of the biofilm formation of *P. aeruginosa* from water vending machine (WVM)

CHAPTER 2

LITERATURE REVIEW

2.1 Water Vending Machine (WVM)

Water vending machine (WVM) is defined as a self-service device that automatically dispenses a set amount of drinking water when appropriate amount of money is inserted and machine vended water means any water that does not come in a sealed container or bottle dispensed by a WVM (Malaysia & International Law Book Services, 2019). These WVM are equipped with a filtration system with different water treatment option such as reverse osmosis, ionization or distillation. The water vended from WVM must go through filtration, treatment and disinfection. Lifestyle changes and modern infrastructure has resulted in the rise in popularity of WVM which provides an accessible and convenient way to obtain clean drinking water. It is usually located at convenient locations such as convenience store, markets, shop lots and a near housing areas. Compared to buying bottled water, WVM are very cost efficient with a set price as low as 10 cents per litre. It is also considered environmentally safe, reducing the use of single-use plastics. Consuming water dispensed by WVM is also regarded as safe as oppose to tap water. Tap water is often associated with a displeasing nickel-like taste.

According to Bennet (2012), tap water can contribute to rash, skin burns, tooth enamel erosion and often contains toxic substances such as lead, barium, arsenic and many other harmful substances in reference to the New York Times investigation. In tap water, there are numerous microorganisms and toxins that have a potential to contribute to allergy and disease symptoms. As a result, the consumers choose to get other alternatives for better quality of drinking water such as bottled water that is commercially sold in the shop, filtered water, water dispenser or the most common choice is WVM. However, the rise in popularity of WVM also arises some concerns. As according to Praveena et al. (2018), drinking water from WVM are not guarantee free from pathogen and harmful bacteria, as there are risks and factors that can contribute to contamination which could affect the standard and safety of the drinking water depending on the maintenance of the machine and the filtration system. Improper maintenance and handling by consumers can lead to cross contamination of the water dispensed.

2.2 Filtration System of Water Vending Machine (WVM)

Water vending machine (WVM) are equipped with a multi-staged filtration and purification system to ensure a clean and safe quality of drinking water. Depending on the make and model of each machine, the type of filtration system can vary. Common type of filtration method used in WVM is sediment filtration, activated carbon filtration, reverse osmosis (RO) filtration, alkali water filtration, and ultraviolet (UV) disinfection. Each filtration method has its advantages and disadvantages, so most WVM have a combination of filtration method to reach a certain quality level of drinking water dispensed. Figures 2.1 shows the general purification process of WVM.

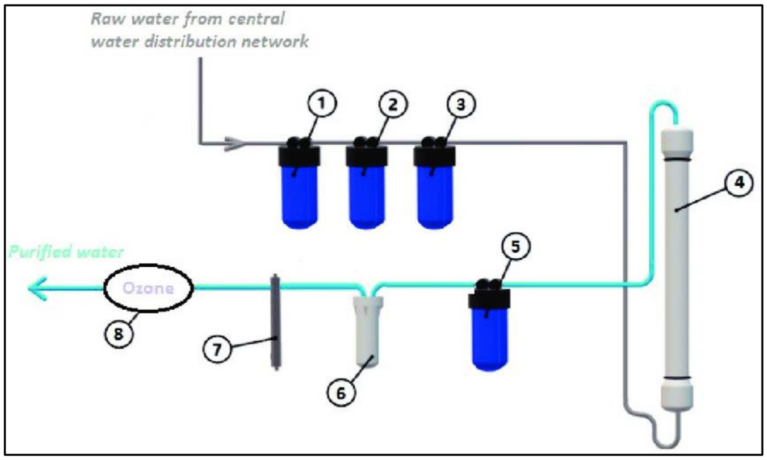

Figure 2.1: General filtration system found in WVM. 1: 5-µg polypropylene sediment filtration cartridge; 2: granular activated carbon cartridge; 3: 1-µg polypropylene sediment filtration cartridge; 4: reverse osmosis membrane stage; 5: granular activated carbon cartridge; 6: calcite-mineralization cartridge; 7: ultraviolet lamp; 8: ozonation (Dėdelė et al., 2022)

2.2.1 Sediment Filtration

Sediment filtration or also known as mechanical filtration is the first initial stage for water filtration is usually sediment filtration to remove large particles present in the water. This method involves physically filtering water by passing it through a filter medium to remove large particles such as sand, silt, clay, loose scale and organic matter (Saha et al., 2023). An example of the type of filters used for this method is mesh filter and ceramic filter. Based on the effectiveness of each filter in term of the particle size it is capable of removing, they are given a micron rating which indicates the size of particles in micrometre the filter is able to remove (Dvorak & Skipton, 2008. The micron rating is usually described as nominal or absolute. Absolute filters can filter approximately 99% of particles larger than its pore size while, nominal filter is can only filter out 90% of particles larger than its pore size (Wu et al., 2021).

2.2.2 Activated Carbon Filtration

Activated carbon filtration is commonly used in water filtration process. It can be used to filter out chlorine, some chemicals, organic compounds and certain heavy metals (Dvorak & Skipton, 2013). Activated carbons are carbons that have been processed to increase its surface area and porosity (Togibasa et al., 2023). Activation can be done through 2 different methods, which are steam activation and chemical activation. During steam activation method, the carbon is first heated in the absence of oxygen, steam is then introduced to activate the carbon (Sharma & Bhattacharya, 2016). Chemical activation involves heating the carbon source at low temperature with the presence of an activating agent (Gañan et al., 2004). The steam activation results in an activated carbon with fine pore structure ideal for adsorbing liquid phase and vapour phase compound (Li et al., 2021). On the other hand, chemical activation results in a greater pore structure making it more suitable for absorbing larger molecules (Nandi et al., 2023). Different material can be used as the carbon source such as bituminous or anthracite coal, bone char, coconut shell and peat. There are 2 types of activated carbon filters, granular activated carbon (GAC) filter and block carbon filter. Granular activated carbon compromises of loose carbon granules, while block carbon filter have been compressed. The GAC filter is used as a polishing filter to remove low concentration compounds usually present in water such as algal toxin, pesticides, industrial micropolutants as well as improving the taste and odour by removing chlorine and hydrogen sulphide (Gabr et al., 2009).

2.2.3 Reverse Osmosis (RO)

Reverse osmosis (RO) is the process of separating solutes in a solution from the solvent by forcing the solvent through a semi permeable membrane to a lower solute concentration by applying external pressure (Ahuchaogu et al., 2018). The pressure required must be more than the natural osmosis pressure to ensure the water travel from the concentrated solution to the diluted solution. The semi-permeable membrane only allows water to pass through, filtering out the dissolved salts, mineral and other contaminants. Table 2.1 shows the type of contaminants RO is able to remove. The water filtered using RO is free from biological contaminants and have low total suspended solids (TSS) and total dissolved solids (TDS) (Lamma et al., 2015). Reverse osmosis is a very efficient method in removing contaminant and producing water with high purity (Kumar & Dixit, 2021). However, there are also a few disadvantages to this method. Reverse osmosis process generates a lot of waste, called reverse osmosis concentrate (ROC) which carries all the contaminants filtered out and it requires proper disposal in order to avoid severe environmental impact (Vaishnav et al., 2023). Other than removing harmful minerals such as lead, RO also removes the beneficial mineral such as iron.

Table 2.1: Contaminants removed by RO (Dvorak & Skipton, 2014).

Ions and metals	Arsenic, Antimony, Aluminium, Barium, Beryllium, Cadmium, Calcium, Chloride, Chromium, Copper, Fluoride, Iron, Lead, Magnesium, Manganese, Mercury, Nitrate, Potassium, Radium, Selenium, Silver, Sodium, Sulphate, Thallium, Zinc
Particles	Asbestos, Protozoa cyst, Cryptosporidium
Pesticides	Endrin, Heptachlor, Lindane, Pentachlorophenol
Radionuclides	Radium, Uranium