Identification of Pathogenic Bacteria Isolated from Raw and After Sand Filtration Water at Lubok Buntar Water Treatment Plant

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ABSTRACT

This study focused on the identification of pathogenic bacteria in raw water intake and after sand filtration for drinking water treatment plant during flood event in 2014. The samples was collected from the Lubok Buntar Water Treatment Plant (WTP) and processed through bacterial isolation using chocolate agar as a media. The isolation process conducted based on serial samples dilution and streaking method prior to DNA extraction. Deoxyribonucleic acid (DNA) extraction kit was used to get selected bacteria DNA and further analysis using Polymerase Chain Reaction (PCR) test and electrophoresis to get DNA sequences. The Basic Local Alignment Search Tool (BLAST) analysis was employed to identify the species of the isolated bacteria. As a result, Pantoeaagglomerans and Enterobacter sp. were found in raw and filtered water sample and indicating the same family types. It was concluded that bacteria of the same species were found before and after sand filtration and need to be removed by disinfectant process. The findings also indicated that all the physicochemical parameters measured were within the values prescribed by the Interim National Water Quality Standard (INWQS).

Keywords: *pathogenic bacteria, bacterial identification, drinking water treatment*

INTRODUCTION

One of the major issues in the developing countries is the water-related disease which comes from bacterial contamination in drinking water supply [1]. The selection of sources of water for drinking water supply usually free from microbial contamination [2]. However, improper sanitary system and urbanisation have led to serious surface water contamination especially related to organic contamination. High load of organics can be source of food for microorganism that can be outbreaks especially during flooding period [3].

Providing good quality of water sources in terms of physical, chemical and bacteriological parameters is a vital to promote safe and economical drinking water treatment system. Disinfection using chlorination has been used as an effective methods for killing bacteria in drinking water system by combining with sedimentation and filtration processes [4, 5]. The removal of classical bacteria such typhoid and cholera are easily can be removed by using this treatment system [6]. Even though water treatment plays an important role by terminating and removing bacteria during the treatment, bacteria still can exist in water even after the treatment or in distribution system. Thus, it is crucial for bacterial identification to classify the pathogenic bacteria that are dominant in water.

According to Heéger *et al.* [7], pathogens like *Salmonella*, *Camphylobactors pp*, *Vibrio cholera, Clostridium botulinim*, *Staphylococcus aureus*, and *Escherichia coli* are the main pathogens that lead to water contamination. In a study conducted by Hussain *et al.* [8], pathogenic bacteria which are *Legionella* and *Pseudomonas aeruginosa* were found in drinking water distribution system of a hospital in Hungary. A study by Kim *et al.,* [9] also addressed a total of ten bacteria species were found in the samples of drinking water. The objective of the study was to determine the contaminations of bacteria in drinking water samples. One of the pathogenic bacteria named *S. aureus* was found in the water sample and it

was believed that the bacteria were responsible for several health issues like wound infection and food spoilage. A study by Mardaneh & Dallal [10] has examined samples from different water sources including bore-well water, tube well water, tap water, and springs. Approximately almost 79 strains bacteria were found existed in the water samples. The study achieved to conclude that *Pseudomonas* followed by *Bacillus* and *Enterobacter* were the most common bacteria found in the samples. This was also agreed by Pindi *et al*. [11] stating that those bacteria are generally the predominant bacteria in the drinking water system.

MATERIALS AND METHOD

Study Area

Water samples were collected from two main sampling points; (i) Raw water sample and (ii) filtered water sample (after sand filtration). The raw water sample was collected from Sungai Kerian at Lubok Buntar, in the district of Bandar Baharu, Kedah. Geographically, the site was located at N 05 07'38.76', E 100 35'42.67'. Total population of Bandar Baharu is 41, 352 people and 1, 921 of the population are from the Lubok Buntar.

Water Sampling and Physicochemical Characteristics Test

Water sampling was conducted on 4th & 18th February 2015 on a sunny day from Lubok Buntar WTP. Figure 1 shows the schematic diagram of Lubok Buntar WTP and the sampling locations. The samples were labelled properly and transported immediately to the laboratory for analyses and stored at a temperature of 4ºC. The physiochemical parameters were conducted based on standard method such as temperature, pH, turbidity, suspended solid (SS), ammonical-nitrogen (NH_3-N), dissolved oxygen (DO), biochemical oxygen demand (BOD) and chemical oxygen demand (COD). The temperature, pH and DO were measured using the YSI multiparameter ORP meter (serial number: 073101344) in situ. Suspended solid measurement was obtained using the HACH DR 2800 and COD test was conducted using the Reactor Digestion Method Calorimetric Determination (spectrophotometer) using the HACH DR 2800. For

the ammoniacal-nitrogen test, the standard Nesslerisation method was employed. COD and SS, for NH_3-N were also obtained using the HACH DR 2800 Spectrophotometer.

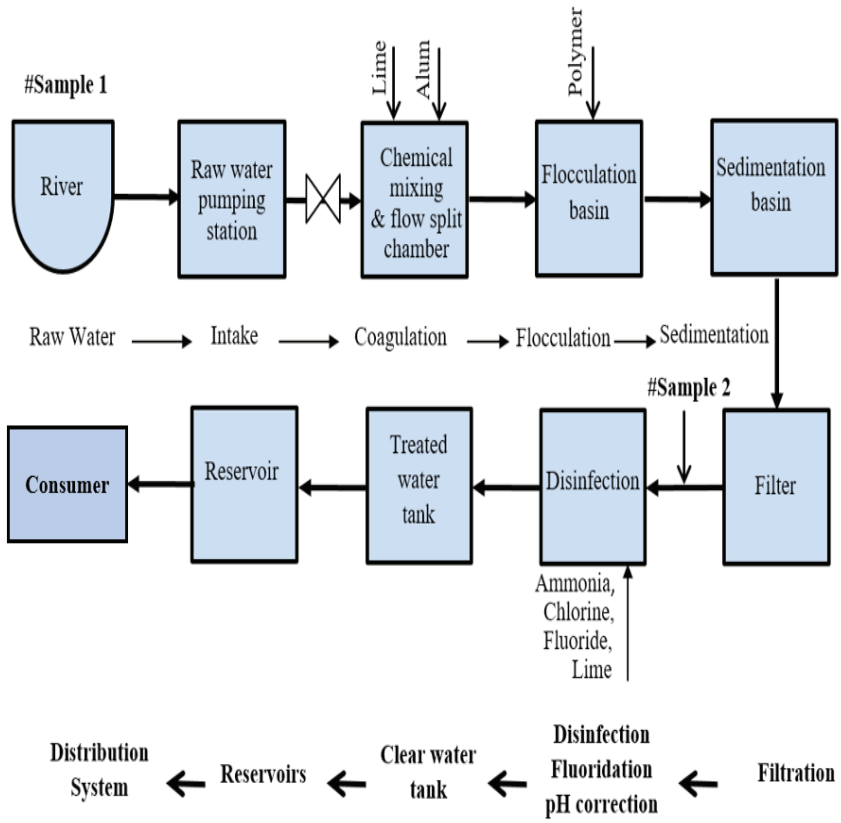

Figure 1: The process flow sheet for Lubuk Buntar WTP

Isolation of Bacterial Cultures

Using inoculation loop, the samples subjected to serial dilution were streaked on chocolate agar for the growth of the bacteria. The plate was then incubated in the incubator for 24 to 48 hours at 37ºC temperature for the bacteria to grow. The grown colonies were examined for their morphology. Gram staining procedure was then performed to group the bacteria into two large groups which were Gram-positive and Gram-negative.

Identification of Bacteria

DNA from the bacteria was extracted using GF-1 Bacterial DNA Extraction Kit. The kit applies the principle where only the DNA is isolated while the cellular proteins, metabolites, salts and other low molecular weight impurities are subsequently removed. The concentration of the extracted DNA was done spectrophotometrically using Nanodrop ®. DNA samples were then preceded for PCR test to amplify the DNA using primers and cycling conditions as presented in Tables 1 and 2.

Electrophoresis of PCR Product and BLAST Analysis

The fragments of the DNA were separated using the electrophoresis where the colour of each fragment was observed and recorded as they passed the detector at the bottom of the gel. The DNA sequences were then constructed based on the colour pattern. After the sequences of the DNA were obtained, they were subjected to the BLAST sequence for similarity search and to identify similar taxa. This was accomplished by comparing the new sequences with sequences that have already been reported and stored in the database maintained by the National Center for Biotechnology Information (NCBI). The names of bacteria isolated were identified using this tool.

Table 1: Sequence of primers

Primer	Sequence of Primers
27 Forward	5'-AGAGTTTGATCMTGGCTCAG-3'
1492 Reverse	5'-GGGTTACCTTGTTACGACTT-3'

Table 2: Polymerase chain reaction cycling conditions

Stage	Temperature (ºC)	Time	Number of Cycles
Initial Denaturation	95	5:00	1
Denaturation	95	0:30	35
Annealing	50	0:30	
Extension	72	1:30	
Final Extension	72	7:00	1
End	4	∞	-

RESULT AND DISCUSSION

The pH of raw water sample was found to be 6.48, while the water sample after sand filtration obtained pH within the range of 6.50-9.00. For temperature, raw water had temperature of 26.50 ºC, whereas 30.80 ºC was attained for water sample after sand filtration. The dissolve oxygen (DO) concentration of raw water was 6.50 mg/L and the other sample was 5.70 mg/L. For turbidity, the raw water sample gave the value of 45 NTU which was higher than that of water sample after sand filtration (0.30 NTU). For chemical oxygen demand (COD) test, raw water sample inhibited 30 mg/L of the COD value, while 27 mg/L was recorded for after sand filtration water sample. For biochemical oxygen demand (BOD) test, raw water sample and water after sand filtration gave the values of 4.51 mg/L and 3.92 mg/L, respectively. Value of ammoniacal-nitrogen (NH_3-N) for raw water sample was 0.52 mg/L and 0.11 mg/L was obtained for water sample after sand filtration.

From the bacteria culture almost five different colonies were discovered on the surface of chocolate agar for the raw water sample, and two different colonies were present after sand filtration water sample. Tables 3 and 4 show the colony and cell morphological of the isolated bacteria for both water samples. From the isolated bacteria, only one bacterium was chosen for each of the samples to obtain the DNA, and the purity of the DNA was quantified spectrophotometrically (Bateria No. 1 for raw water sample and Bacteria No. 2 for after sand filtration water sample). Both DNAs were considered as free from protein because both ratios of A_{260}/A_{280} and A_{260}/A_{230} were between 1.8 and 2.0. The DNAs were then proceeded for PCR and electrophoresis test where the DNA sequences were obtained. The BLAST analysis was also used to further validate the identification of bacterial isolates. As a result, it was concluded that *Pantoeaagglomerans*is was found in raw water sample, while *Enterobacter sp.* was found in after sand filtration water sample.

Table 3: Colony and cell morphologies of bacteria isolated from raw water sample

Bacteria No.	Gram Staining	Colony Morphology
1.	Pink (-ve bacilli)	Form: Circular Elevation: Raised Margin: Entire Opacity: Translucent Pigmentation: No pigmented
2.	Purple (+ve bacilli)	Form: Circular Elevation: Flat Margin: Entire Opacity: Opaque Pigmentation: Whitish
3.	Purple (+cocci)	Form: Irregular Elevation: Flat Margin: Undulate Opacity: Opaque Pigmentation: Whitish
4.	Purple (+ve bacilli)	Form: Circular Elevation: Convex Margin: Undulate Opacity: Opaque Pigmentation: Whitish
5.	Pink (-ve coccobacilli)	Form: Irregular Elevation: Raised Margin: Undulate Opacity: Opaque Pigmentation: Whitish

Table 4: Colony and cell morphologies of bacteria isolated from after sand filtration water sample

Bacteria No.	Gram Staining	Colony Morphology
1.	Pink (-ve cocci)	Form: Circular Elevation: Flat Margin: Entire Opacity: Opaque Pigmentation: Whitish
2.	Purple (+ve bacilli)	Form: Irregular Elevation: Umbonate Margin: Undulate Opacity: Transparent Pigmentation: Cream colour

Two different water samples namely raw water and water after sand filtration were collected from Lubok Buntar WTP. Raw water sample yielded about five isolates, while the after sand filtration water sample yielded two isolates. Of that total, only one isolate was selected for further analysis. Based on the morphology, both organisms were Gram-negative bacteria of the *Enterobacteriaceae* family. Thus, both organisms should have similar characteristics since they belonged to the same family. The bacteria cell has a bacillus (rod) shape, non-sporming bacteria and classified as pathogenic bacteria for both human and animals [12]. *P. Agglomerans* and other *Pantoea*genus may contribute to urinary tract infection in human which is commonly isolated from human, soils, water, animals and plants. During the bacterial culture, *P. Agglomerans* also produces beta-hemolysis where it has the capability to breakdown the hemoglobin or red blood cell in the culture media (blood culture).

Commonly, this organism can be classified as an opportunistic pathogen because of its low degree of toxicity and low virulence, but may infect any organs of human and animals. However, its pathogenicity and virulence are difficult to ascertain and reveal [12]. In Europe, according to Van Rostenberghe *et al.* [13], *P. Agglomerans* has been classified as species that has biosafety level 2 (BL-2) organisms and can cause synovitis or septic arthritis. As reported by De Boeck *et al.* [3], in their study also highlighted the two strains of *Enterobacteriacea* in drinking water sources. Similar to *P. Agglomerans,* the *Enterobacter sp.* mostly existence in the environment

of soils, sewage,and water. It can inhibit the intestines of animals and human. These organisms are rarely known as pathogen, but considered as opportunistic pathogens and are recently encountered more often [14]. It has also been often implicated in wound and urinary tract infections which always cause meningitis and septicemia. Similar to other pathogenic bacteria, *Enterobacter sp.* is enriched with virulence factors which are adhesions, endotoxin and siderophones to acquire iron. *Enterobacter sp* can also cause health effects such as skin infections, bacteremia, and pneumonia. Drinking water that contains *Enterobacter sp* directly can cause diarrhea which is the common problem in United States, and the presence of pathogens in drinking water may increase the risk of developing infections among consumers [15].

The mixed acid pathway is the main pathway of *Enterobacteriaceae* where produce the end products includes of lactic acid, ethanol, formic acid andacetic acid. It has the possibility to form carbon dioxide and hydrogen if the bacterium possesses the formatedehyrogenase enzyme. They are also catalase positive and usually reduce nitrate to nitrite. Formic acid, acetic acid and lactic acid are weak acids that have numerous applications in biotechnology. If the production of mixed acid is high, it can affect the water quality which can lower the pH of water. Low pH can give undesirable effect on plumbing and piping. The water treatment plant has disinfection process that kills the microorganism contained in water. However, the quality of water may differ during the treatment. In this study, it can be said that bacteria found in raw water (before treated) are also found in water after sand filtration because both of them belonged to the same family. *Enterobacteriaceae* family is known as Gram-negative bacteria which when entering the water distribution system will contribute to the biofilm formation. The biofilm formation is developed when bacteria adhere to the water surface and starts to excrete sticky stuff to stick materials like soil particles, plastics and most significantly, the animal or human tissues. This biofilm subsequently can preserve microbes from disinfection. Although disinfection (chlorination) is the process applied in water treatment to kill bacteria, the lipopolysaccharides (LPD) is the outer membrane of Gram-negative wall that shield the membrane from being attacked by the chemical. It can be concluded that, even after the process of treatment (before disinfection), the pathogenic bacteria were still found, meaning that microorganisms were not well filtered.

CONCLUSION

This investigation concluded the existence of pathogenic bacteria in both water samples. In the raw water sample, the bacterium found was *Pantoea Agglomerans*, while for the water sample from after sand filtration was *Enterobacter sp.* The characteristic of both bacteria showed hemolysis criteria where they have the ability to destroy red blood cells. The bacteria also produced mixed acid from glucose which has numerous applications in biotechnology. Although bacteria are reduced in the treatment process, they might still survive after the treatment process. Therefore, boiling water is strongly recommended before consuming water.

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