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Molecular profiling of culturable bacteria from portable drinking water filtration systems and tap water in three cities of Metro Manila, Philippines

Edward A. Barlaan*, Janina M. Guarte, and Chyrene I. Moncada

Molecular Diagnostics Laboratory, Institute of Biology, College of Science, University of the Philippines, Diliman, Quezon City, 1101, Philippines

Abstract—Many consumers drink filtered water from portable filtration system or directly from tap water. However, microbial community composition in portable drinking water filtration systems has not yet been investigated. This study determined the molecular profile of culturable bacteria in biofilms and filtered water from portable drinking water filtration systems and tap water in three key cities of Metro Manila, Philippines. A total of 97 isolates were obtained using different growth media and characterized based on 16S rRNA gene sequences. Most bacteria were isolated from biofilms, followed by filtered water and the least from tap water. Many isolates were affiliated with Proteobacteria (α, β, and γ), Actinobacteria, Firmicutes and Bacteriodetes; some had no matches or low affiliations in data bank. Many isolates were associated with bacteria that were part of normal drinking water flora. Some were affiliated with opportunistic bacterial pathogens, soil bacteria and activated sludge bacteria. The presence of soil and opportunistic bacteria may pose health risks when immunocompromised consumers directly drink the tap water. Some isolates had very low percentage homology with bacterial affiliates or without matches in the data bank suggesting different identities or novelty of the isolates. Further studies are needed for different portable filtration systems available in the market, drinking water quality status of other areas and functions of the isolated bacteria. This study is the first report on the use of potato dextrose agar for isolation of bacteria from water environment. It is also the first report on molecular profiling of culturable bacteria in biofilms and filtered water in portable drinking water filtration systems.

Keywords—microbial community, drinking water, portable filtration systems, biofilms, water flora, 16S rRNA

INTRODUCTION

Safe drinking water is a necessity for human and other domestic consumption. While many consumers drink bottled water, others use tap water directly or drink the filtered water from portable filtration system. Some consumers are reluctant to drink tap water directly due to experiences or reported cases of stomach or diarrheal problems. Thus, some households install portable filtration systems as source of drinking water. However, no studies have been reported yet on the bacterial community composition of the drinking water portable filtration systems. Therefore, there is a need to investigate the composition of cultivable bacteria from biofilm deposits on filters and filtered water from the system in comparison with tap water. Although drinking water source has originally undergone treatment, the distribution systems and installations may have inadvertently introduced possible sources of contamination. Therefore, the bacteria found on ceramic filters of the portable filtration system would reflect the strains or species that would have been taken in directly by the consumers using the tap water.

Waterborne diseases in many urban and rural areas in Philippines remain a severe public health concern. Over 500,000 diarrheal diseases with an estimate of more than 13,000 deaths are reported annually in the Philippines (Hutton et al., 2008). The challenges in the country include limited access to sanitation and in particular to sewers; high pollution of water resources; often poor drinking water quality and poor service quality (ADB, 2007; ADB, 2013). Poor sanitation infrastructure, fluctuations in water pressure caused by power outages, leaky pipes and cross connections between drinking water and sewage systems lead to introduction of waterborne pathogens and water contamination (Kumpel & Nelson, 2013). Drinking water distribution systems are, therefore, compromised and become vulnerable to biological contamination (Fawell & Nieuwenhuijsen, 2003; Emmanuel et al., 2009). Drinking water pathogens that cause serious problems include bacteria such as *Salmonella, Campylobacter, Shigella, Vibrio, Escherichia, Yersinia, Legionella*), protozoa including *Entamoeba, Naegleria, Cryptosporidium, Giardia*) and viruses like Norovirus, Rotavirus, Adenovirus (Ashbolt, 2004).

Various technologies have been used to detect and analyze pathogens in drinking water including traditional microscopic techniques, biochemical tests, culture-based method, membrane filtration, multiple tube method and the most probable number method (WHO, 2011). Other methods include biosensors (Wilkins et al., 1999), *in-situ* hybridization techniques, immunological techniques (Servais et al., 2002) and immunomagnetic-electrochemiluminescent techniques (Yu & Bruno, 1996). However, these methods are either time-consuming, expensive or have low sensitivity (O'Kennedy et al., 2003; Alain & Querellou, 2009; Zengler, 2009). Alternatively, polymerase chain reaction (PCR) technology is a rapid, highly-sensitive and cost-effective method to detect a range of

*Corresponding Author
Email Address: edward.barlaan@up.edu.ph
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waterborne pathogens in a single water sample (Tissier et al., 2012). Pyrosequencing is another molecular approach for comprehensive understanding of microbial community and diversity in drinking water (Navarro-Noya, et al. 2013; Liu et al., 2014). Despite the limitation, culture-dependent technique continues to be practically useful as it provide researchers with live bacteria that can be conserved as pure cultures and repositories of microbial strains. It also allows practical and direct approach in assessing environmental samples, particularly in detection of contaminants, pathogens and economically important strains in soil, water or other environments. In bacteria, 16S rRNA gene is commonly used and is a very powerful tool in identification.

This present study aimed to isolate bacteria from ceramic filter biofilms and filtered water of portable drinking water filtration systems and tap water; to identify the isolated bacteria using 16S rRNA gene sequencing; to determine associations of isolates with other bacteria available in nucleotide data bank; and to assess phylogenetic relationships among the isolates collected from water samples in three cities in Metro Manila.

MATERIALS AND METHODS

Sample collection

Metro Manila derives its water from the Angat Dam about 40 km to the Northeast of Manila. The water flows through the Angat River to smaller Ipo Dam where the water is diverted through tunnels to La Mesa Basins. From these basins, water is provided to West Manila and East Manila, where the two private water companies treat the raw water. The water treatment processing for East Manila water supply involves chlorination, coagulation, flocculation, passage to settling basins, filter beds and chlorine gas injection before it reaches the taps. Three cities namely, Quezon City, Marikina City and Mandaluyong City were selected for sampling to represent the East Manila. Samples were collected from an office in the University of the Philippines in Quezon City, from a residential kitchen in Barangay Barangka, Marikina City, and from a kitchen in Rizal Technological University in Mandaluyong City, Philippines. New portable drinking water filtration system (Megafresh Ceramic Water Filter System, Shen Hung Enterprise Co., Taiwan) was installed in each site. The portable drinking water filtration system had two cartridges: (a) cartridge with 0.9-μm ceramic water filter and (b) water filter cartridge with granular activated carbon and silver. After one-week use, 100-ml samples were collected each from tap water, filtered water and biofilms on ceramic filter chamber. Chlorinated tap water samples were obtained directly from the faucet. Filtered water was collected from portable drinking water filtration system. Biofilm microbial samples were scraped off the ceramic filter chamber and washed with sterile water. Samples were collected in sterile 100-ml reagent bottles and processed immediately after collection.

Bacterial culture and isolation

One hundred microliters (100 μl) of each tap water, filtered water and biofilm samples were inoculated on sterilized nutrient agar (NA), potato dextrose agar (PDA) and trypticase soy agar (TSA) (HiMedia Laboratories, India) using spread plate method. Preparations for each culture medium were made in three replicates. All growth media were prepared according to the instructions of manufacturers. Culture plates were incubated at 37°C for 48 hrs. Colonies were carefully selected based on distinct morphological differences such as colony shape, margin, elevation, color and diameter described by Brown (2007). Isolates were further purified via the streak plate method. In cases when two or more isolates with exact similar morphological characters within the same culture medium and sample source and showed exactly the same DNA sequences and homologies in BLAST analysis, only one isolate was considered for analysis to avoid duplication.

DNA extraction

DNA of each bacterial isolate was extracted using Zymo Research Fungal/ Bacterial DNA MiniPrep (ZymoResearch Corporation, USA) according to the manufacturer's protocol with minor modifications. Vortexing was increased to 15 min instead of 5 min for cell lysis. DNA extracts were eluted in 100 μl DNA Elution Buffer (ZymoResearch). DNA purity and yield were determined using the QUANTUS Fluorometer (Promega, USA).

PCR amplification and gel electrophoresis

Amplification of the 16S rRNA gene was carried out on a Veriti® 96-Well Thermal Cycler (Thermo Fisher Scientific Inc., USA) with a 25 μl reaction mixture containing 50 ng of DNA, 2.5 μl 10X PCR buffer (Clonetech Laboratories, USA), 1 μl 50 mM $MgSO_4$(Clonetech), 0.5 μl 10 mM dNTP mixture (Invitrogen Life Technologies Corporation, USA), 10 μM each of forward primer (27F: AGAGTTTGATCMTGGCTCAG) and reverse primer (1492R: GGYTACCTTGTTACGACTT) (Weisburg et al., 1991; Long & Azam, 2001), 0.5μl Titanium Taq Polymerase (Clonetech) and 38.8 μl PCR-grade water. The PCR program was set as follows: an initial denaturation step at 95°C for 1 min; 25 cycles at 95°C for 30 s, 60°C for 30 s, 72°C for 30 s, followed by a final extension at 72°C for 3 min. Amplicons were analyzed on 0.8% agarose gel stained with GelRed (Biotium, Hayward, CA) in 0.5X TAE buffer run at 100 V for 30 min. A 100-bp ladder (Vivantis Technologies, Selangor, Malaysia) was used as molecular weight marker. The gels were visualized under LED transilluminator (Maestrogen, Nevada, USA) and photographed using Alpha Imager Mini Imaging System (ProteinSimple, USA).

Sequence and data analysis

Amplicons obtained from the 16S rRNA gene amplification were sent to First BASE Laboratories (Malaysia) for sequencing. Sequences were searched against the GenBank database through the BLASTN program of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST) to identify closest matches. To determine the distribution of bacterial isolates in sampling locations, culture growth media and isolation sources, Venn diagrams were created using Venn Diagram Generator (2015). The nucleotide sequences were aligned using ClustalW of the MEGA 6 software (Tamura et al., 2013). The phylogenetic tree was constructed using the Maximum-Likelihood (ML) method. Genetic distances were computed using Kimura 2-Parameter (K2+G) model with 1000 bootstrap replicates. *Methanopyrus kandleri,* an archaeal methanogen, was rooted as outgroup due to its distant affinity with bacteria.

RESULTS

Bacterial isolates

A total of 97 bacterial isolates were obtained from different culture growth media, isolation sources and three cities (Table 1). There were 57, 30 and 10 bacteria isolated from NA, TSA and PDA, respectively (Table 1A). Most of bacterial species or strains grew in specific medium; few strains thrived also in different medium. The strains that grew both in NA and TSA were *Stenotrophomonas* sp. Hy3tC5 (MK-BN4 and MD-BT1), *Delftia* sp. C-5 (MD-BN5, MD-FT1 and MD-BT3) and *Pseudomonas* sp. SBR25 (Q-BN3 and Q-BT3) while in NA and PDA was *Pseudomonas* sp. Cf0-4 (Q-BN2, Q-BP2 and Q-FP1) (Fig. 1A). There were more bacteria isolated in biofilms (n=63), followed by filtered water (n=31) and the least in tap water (n=3) (Table 1B). While most of the bacteria species or strains were isolated in specific isolation source, there were strains that were found in both biofilms and filtered water. These include *Pseudomonas* sp. Cf0-4 (Q-BN2, Q-BP2 and Q-FP1), *Pseudomonas sp*. J4AJ (Q-BT1, Q-FT1, *Acinetobacter* sp. A17 (MD-BN4, MD-FN7), *Delftia* sp. C-5 (MD-BN5, MD-BT3 and MD-FT1) and *Cupriavidus* sp. TSA49 (Q-BP3 and MD-FP1) (Fig. 1B). Among the three cities, Quezon City had 19, Marikina City had 35 and Mandaluyong City had 43 isolates (Table 1). Most of the bacterial strains were exclusive to the sampled city. However, a few strains were commonly found in Mandaluyong City and Quezon City like *Cupriavidus* sp. TSA49 (Q-BP3 and MD-FP1) and in Marikina City and Mandaluyong City such as *Stenotrophomonas* sp. Hy3tC5 (MK-BN4 and MD-BT1). Based on 16S rRNA gene sequence and BLAST analyses, isolates were associated with bacteria belonging to Proteobacteria (α, β, and γ), Actinobacteria and Firmicutes and uncultured clones. Some isolates had no significant matches or associates in the nucleotide data bank (Fig. 2).

TABLE 1. Number of bacterial isolates in different (A) culture media and (B) isolation sources from three cities of Metro Manila.

	Quezon City	Marikina City	Mandaluyong City	Total
A. Culture Medium				
NA	9	24	24	57
TSA	5	10	15	30
PDA	5	1	4	10
Total	19	35	43	97
B. Isolation Source				
Biofilm	12	19	32	63
Filtered water	6	15	10	31
Tap water	1	1	1	3
Total	19	35	43	97

NA= Nutrient Agar; TSA= Trypticase Soy Agar; PDA= Potato Dextrose Agar

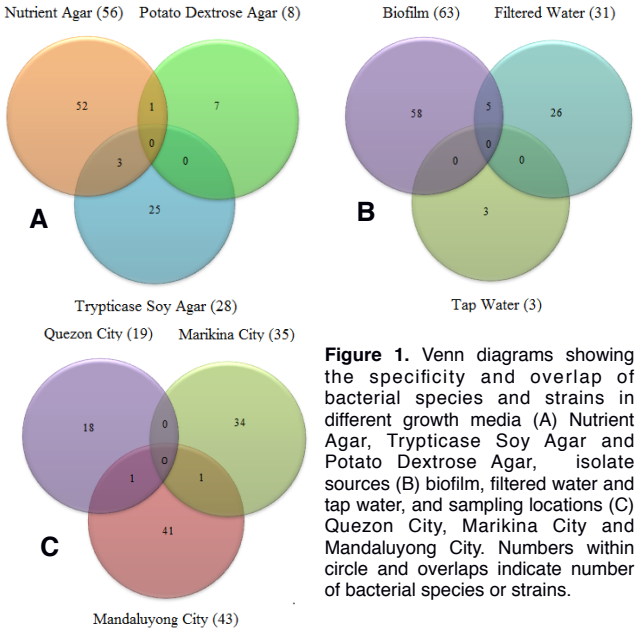

Figure 1. Venn diagrams showing the specificity and overlap of bacterial species and strains in different growth media (A) Nutrient Agar, Trypticase Soy Agar and Potato Dextrose Agar, isolate sources (B) biofilm, filtered water and tap water, and sampling locations (C) Quezon City, Marikina City and Mandaluyong City. Numbers within circle and overlaps indicate number of bacterial species or strains.

TABLE 2. Bacterial isolates from three cities of Metro Manila, biofilms and filtered water of filtration systems and tap water with different information of the closest match in NCBI data bank.

Isolate*	Closest Match	Homology	GenBank Accession No.	Taxonomic Classification	Sources/Roles of Associated Bacteria
Quezon City					
Q-BN1	*Pseudomonas otitidis* strain T3946d	92%	DQ298037.1	γ- proteobacteria	otitis infection
Q-BN2	*Pseudomonas* sp. Cf0-4	92%	JN836274.1	γ- proteobacteria	ether enrichment culture
Q-BN3	*Pseudomonas* sp. SBR25	96%	KC936277.1	γ- proteobacteria	sludge reactor
Q-BN4	Bacterium clone ncd1843d02c1	82%	JF158641.1	-	skin microbiome
Q-BP1	*Ralstonia* sp. NAH8	81%	AF361022.1	β- proteobacteria	PAHs biodegradation
Q-BP2	*Pseudomonas* sp. Cf0-4	94%	JN836274.1	γ- proteobacteria	ether enrichment culture
Q-BP3	*Cupriavidus* sp. TSA49	88%	AB542403.1	β- proteobacteria	oligotrophic denitrifier
Q-BP4	Bacterium clone nbw97e10c1	89%	GQ008855.1	-	skin microbiome
Q-BT1	*Pseudomonas* sp. J4AJ	92%	KF668329.1	γ- proteobacteria	club-rush endophyte
Q-BT2	*Pseudomonas* sp. clone B3-51	88%	KF448083.1	γ- proteobacteria	petroleum reservoirs
Q-BT3	*Pseudomonas* sp. SBR25	94%	KC936277.1	γ- proteobacteria	sequencing batch reactor
Q-BT4	Bacterium clone ncd1521h06c1	83%	JF132930.1	-	skin microbiome
Q-FN1	*Pseudomonas* sp. P7(2009b)	87%	GU113077.1	γ- proteobacteria	lake water
Q-FN2	*Pseudomonas* sp. BND-BHI2	86%	HQ613832.1	γ- proteobacteria	mosquito midgut
Q-FN3	Bacterioplankton clone E305B_42	94%	KC002489.1	-	seawater surface
Q-FN4	*Kocuria* sp. 97H2c	86%	KJ744013.1	Actinobacteria	soil
Q-FP1	*Pseudomonas* sp. Cf0-4	89%	JN836274.1	γ- proteobacteria	ether enrichment culture
Q-FT1	*Pseudomonas* sp. J4AJ	97%	KF668329.1	γ- proteobacteria	club-rush endophyte
Q-TN1	*Brachybacterium* sp. clone iso5_d	78%	EU175952.1	Actinobacteria	salt-secreting desert tree
Marikina City					
MK-BN1	*Kocuria rhizophila* strain DC2201	97%	NR_074786.1	Actinobacteria	soil
MK-BN2	Bacterium clone B38	96%	HQ697426.1	-	activated carbon
MK-BN3	No significant matches	-	-	-	-
MK-BN4	*Stenotrophomonas* sp. Hy3tC5	97%	KJ563080.1	γ- proteobacteria	bark beetle
MK-BN5	*Staphylococcus arlettae* strain LCR34	98%	FJ976543.1	Firmicutes	paddy field soil
MK-BN6	Bacterium clone nck177d11c1	96%	KF093565.1	-	skin
MK-BN7	Bacterium clone nbw158b08c1	85%	GQ073811.1	-	skin microbiome
MK-BN8	Bacterium clone ncd2424c04c1	81%	JF210534.1	-	skin microbiome
MK-BN9	*Pseudomonas alcaligenes* strain B2M2O	84%	JN644066.1	γ- proteobacteria	river biofilm
MK-BN10	No significant matches	-	-	-	-
MK-BN11	No significant matches	-	-	-	-
MK-BP1	Bacterium clone nbw1134b02c1	87%	GQ080114.1	-	skin microbiome
MK-BT1	No significant matches	-	-	-	-
MK-BT2	Bacterium clone ncm71c06c1	73%	KF108464.1	-	skin
MK-BT3	Bacterium clone nck307a03c1	96%	KF106088.1	-	skin
MK-BT4	Bacterium clone nbw665f06c2	89%	GQ111345.1	-	skin microbiome
MK-BT5	Bacterium clone nbw168a02c1	74%	GQ075084.1	-	skin microbiome
MK-BT6	No significant matches	-	-	-	-
MK-BT7	No significant matches	-	-	-	-
MK-FN1	*Chryseobacterium* sp. AG1-2	86%	KC560016.1	Bacteroidetes	moss
MK-FN2	No significant matches	-	-	-	-
MK-FN3	*Barrientosiimonas humi* strain 39	96%	NR_126227.1	Actinobacteria	soil
MK-FN4	No significant matches	-	-	-	-
MK-FN5	No significant matches	-	-	-	-
MK-FN6	*Pseudoxanthomonas japonensis* ZKB-2 4-4	78%	KC464453.1	γ- proteobacteria	soil
MK-FN7	No significant matches	-	-	-	-
MK-FN8	No significant matches	-	-	-	-
MK-FN9	No significant matches	-		-	-
MK-FN10	Alpha proteobacterium clone O-5-58	87%	KF827248.1	α- proteobacteria	biofilm
MK-FN11	Enrichment culture clone Lpl3-225	83%	JX963071.1	β- proteobacteria	landfill
MK-FN12	No significant matches	-	-	-	-
MK-FT1	*Agrobacterium tumefaciens* strain E3-2	95%	JX110584.1	α- proteobacteria	sea sediment
MK-FT2	*Rhizobium massiliae* strain CCNWQLS35	82%	JX840367.1	α- proteobacteria	soil legumes
MK-FT3	*Staphylococcus* sp. M5-7-5	81%	FJ832082.1	Firmicutes	fish sauce
MK-TN1	No significant matches	-	-	-	-
Mandaluyong City					
MD-BN1	Bacterium clone P3D1-451	93%	EF509146.1	-	endotracheal aspirate
MD-BN2	*Stenotrophomonas maltophilia* strain Ysm	93%	KF278963.1	γ- proteobacteria	crowdipper endophyte
MD-BN3	*Pseudomonas putida* strain JC186	90%	KJ534476.1	γ- proteobacteria	*Triticum* sp. endophyte
MD-BN4	*Acinetobacter* sp. A17	97%	JX195717.1	γ- proteobacteria	activated sludge
MD-BN5	*Delftia* sp. C-5	96%	KC702840.1	β- proteobacteria	soil
MD-BN6	*Pseudomonas aeruginosa* strain SMVIT-1	93%	KJ671465.1	γ- proteobacteria	oil spill soil sample
MD-BN7	*Ponticoccus gilvus* strain 19-DR	95%	JN712171.1	Actinobacteria	bark beetle gut
MD-BN8	*Micrococcus luteus* strain LHR-04	89%	HE716930.1	Actinobacteria	reed necrotic lesion
MD-BN9	*Pseudomonas aeruginosa* strain SZH16	93%	GU384267.1	γ- proteobacteria	phenol-degradation
MD-BN10	*Pseudomonas aeruginosa* strain L1R2-2	96%	EU139850.1	γ- proteobacteria	soil
MD-BN11	*Pseudomonas aeruginosa* strain L1R2-3	94%	EU139850.2	γ- proteobacteria	soil
MD-BN12	*Pseudomonas aeruginosa* strain MBL	79%	KF811604.1	γ- proteobacteria	*Clarias gariepinus* lesion
MD-BN13	Bacterium clone L3-B53	93%	KJ548976.1	-	hydrothermal field water
MD-BN14	Bacterium clone P7D1-424	82%	EF509404.1	-	endotracheal aspirate
MD-BN15	*Bacillus pumilus* strain FeRB-FL1404	93%	KM405294.1	-	soil
MD-BN16	*Pseudomonas* sp. SSKSD6	98%	KF751674.1	Firmicutes	river

MD-BP1	Bacterium clone ncd2190f07c1	94%	JF190687.1	-	skin microbiome
MD-BP2	*Vibrio* sp. 442	93%	AB081772.1	γ- proteobacteria	Japanese flounder
MD-BP3	*Pseudomonas* sp. RPT-52	96%	JF756593.1	γ- proteobacteria	soil
MD-BT1	*Stenotrophomonas* sp. Hy3tC5	95%	KJ563080.1	γ- proteobacteria	beetle
MD-BT2	*Stenotrophomonas maltophilia* strain BBE11-1	95%	JQ619623.1	γ- proteobacteria	keratin wastes biodegradation
MD-BT3	*Delftia* sp. C-5	98%	KC702840.1	β- proteobacteria	soil
MD-BT4	*Pseudomonas aeruginosa* strain F4	91%	HF572851.1	γ- proteobacteria	soil
MD-BT5	*Pseudomonas aeruginosa* strain GiTPA	92%	FJ823152.1	γ- proteobacteria	oral cavity
MD-BT6	*Pseudomonas aeruginosa* strain HOB1	89%	EU849119.1	γ- proteobacteria	canal sediment
MD-BT7	*Pseudomonas aeruginosa* isolate D2	93%	LK391633.1	γ- proteobacteria	soil
MD-BT8	Bacterium clone nbw227d02c1	91%	GQ068996.1	-	skin microbiome
MD-BT9	*Proteus mirabilis* strain SZH18	88%	GU384269.1	γ- proteobacteria	phenol-degradation
MD-BT10	Bacterium clone 16sps26-3h01.p1ka	83%	FM997495.1	-	sputum
MD-BT11	*Pseudomonas aeruginosa* strain PGPR 10	87%	KF640236.1	γ- proteobacteria	tomato root surface
MD-BT12	*Pseudomonas* sp. LJLP1-15	90%	DQ140184.1	γ- proteobacteria	biofilm
MD-BT13	Pseudomonadaceae bacterium ALE_F7	93%	GQ128373.1	γ- proteobacteria	activated carbon filters
MD-FN1	*Delftia* sp. X-a12	94%	JX997845.1	β- proteobacteria	oil production water
MD-FN2	Bacterium clone FPURT2-B01	79%	GU166615.1	-	citrus roots/ rhizospheres
MD-FN3	Bacterium clone ncd759d10c1	89%	HM295740.1	-	skin microbiome
MD-FN4	Bacterium clone nbw124g01c1	92%	GQ024639.1	-	skin microbiome
MD-FN5	*Pseudomonas aeruginosa* isolate L1	92%	LK391632.1	γ- proteobacteria	soil
MD-FN6	*Microbacterium oleivorans* strain DSR8	96%	JQ342859.1	Actinobacteria	soil
MD-FN7	*Acinetobacter* sp. A17	97%	JX195717.1	γ- proteobacteria	activated sludge
MD-FP1	*Cupriavidus* sp. TSA49	94%	AB542403.1	β- proteobacteria	oligotrophic denitrifier
MD-FT1	*Delftia* sp. C-5	95%	KC702840.1	β- proteobacteria	soil
MD-FT2	*Micrococcus luteus* strain SCAU-A-105	95%	KJ671455.1	Actinobacteria	salt lake in China
MD-TN1	Bacterium clone ncd1554h06c1	94%	JF135142.1	-	skin microbiome

*Q= Quezon City; MK= Marikina City; MD= Mandaluyong City; B= Biofilm; F= Filtered water; T= Tap water; N= Nutrient Agar; T= Trypticase Soy Agar; P= Potato Dextrose Agar.

Bacterial community composition in Quezon City

Majority of bacterial isolates (63%) from Quezon City were affiliated to Phylum Proteobacteria (Table 2). About 53% belonged to γ Proteobacteria particularly to genus *Pseudomonas* (Q-BN1, Q-BN2, Q-BN3, Q-BP2, Q-BT1, Q-BT2, Q-FN1, Q-FN2, Q-FP1 and Q-FT1) and 10% to β Proteobacteria specifically to genera *Ralstonia* (Q-BP1) and *Cupriavidus* (Q-BP3). Other isolates belonged to Phylum Actinobacteria particularly to genera *Kocuria* (Q-FN4) and *Brachybacterium* (Q-TN1) and some were associated with unidentified and uncultured bacterial clones (Q-BN4, Q-BP4, Q-BT4 and Q-FN3). In comparison with other cities, the genera *Ralstonia* and *Brachybacterium* were exclusively found in Quezon City.

There were 12 isolates obtained from biofilm, which were associated with genera *Pseudomonas, Ralstonia, Cupriavidus* and uncultured clones; six isolates from filtered water, which were related to *Pseudomonas*, *Kocuria* and uncultured clones; and one from tap water with close association to *Brachybacterium* (Table 1B, Table 2). Except Q-FT1, most isolates had relatively low percentage homology (78- 96%) with their associates.

Bacterial community composition in Marikina City

Most of the bacterial isolates in Marikina City (66%) were associated with uncultured bacterial clones or without significant matches found in data bank (Table 2). With known associates, isolates were related to Proteobacteria (MK-BN4, MK-BN9, MK-FN6, MK-FN10, MK-FN11, MK-FT1 and MK-FT2) specifically to genera *Stenotrophomonas, Pseudomonas, Pseudoxanthomonas, Agrobacterium* and *Rhizobium*; Actinobacteria (MK-BN1 and MK-FN3) to genera *Kocuria* and *Barrientosiimonas*; and Firmicutes (MK-BN5 and MK-FT3) to *Staphylococcus*. Genera *Staphylococcus, Chryseobacterium, Barrientosiimonas, Pseudoxanthomonas, Agrobacterium* and *Rhizobium* were exclusively found in Marikina.

There were relatively more isolates obtained in biofilms (19) than in filtered water (15) while only one isolate in tap water (Table 1B). Isolates from biofilm were related to genera *Kocuria, Stenotrophomonas, Staphylococcus, Pseudomonas* and mostly uncultured bacterial clones or without matched associates (Table 2). Bacterial isolates from filtered water were affiliated with *Chryseobacterium, Barrientosiimonas, Pseudoxanthomonas, Agrobacterium*, *Staphylococcus* and uncultured clones or unknown associates. Isolate from tap water had no known relative in the data bank. Except for isolates MK-BN1, MK-BN4 and MK-BN5, all other isolates had relatively lower percentage homology (73-96%).

Bacterial community composition in Mandaluyong City

Many bacterial isolates were related with Proteobacteria (64%) in Mandaluyong City (Table 2). Among the three cities, it comprised more genera under Proteobacteria such as *Stenotrophomonas* (MD-BN2, MD-BT1 and MD-BT2), *Pseudomonas* (MD-BN3, MD-BN6, MD-BN9 – MD-BN12, MD-BP3, MD-BT4 – MD-BT7, MD-BT9, MD-BT11 – MD-BT13 and MD-FN5), *Acinetobacter* (MD-BN4 and MD-FN7), *Delftia* (MD-BN5, MD-BT3, MD-FN1 and MD-FT1), *Vibrio* (MD-BP2), *Proteus* (MD-BT9) and *Cupriavidus* (MD-FP1). Other bacterial isolates belonged to Actinobacteria including genera *Ponticoccus* (MD-BN7), *Micrococcus* (MD-BN8) and *Micrococcus* (MD-FT2) and Firmicutes with genus *Bacillus* (MD-BN16). Genera *Ponticoccus, Micrococcus, Bacillus, Proteus, Vibrio, Microbacteriumo* and *Micrococcus* were exclusively found in Mandaluyong City.

There were 32 bacteria isolated from biofilm, 10 from filtered water and 1 from tap water (Table 1B). Isolates from biofilm were associated with one or more species of *Pseudomonas, Stenotrophomonas, Acinetobacter, Delftia, Vibrio* and *Proteus* (Table 2). Different strains of *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* were notably observed. In filtered water, bacterial isolates were related to *Delftia, Pseudomonas, Microbacterium, Acinetobacter, Cupriavidus* and uncultured clones. Isolate from tap water was associated with uncultured bacterial clone. Except for MD-BN4, MD-BN17, MD-BT3 and MD-FN7, isolated bacteria had relatively lower % homology (79- 96%) to the associated bacteria.

Phylogenetic analysis

Phylogenetic analysis revealed that most of bacterial isolates generally clustered according to the city where they were sampled (Fig. 2). However, some isolates were found to cluster with other locations or phyla particularly those isolates associated with uncultured bacterial clones, without significant matches in the data bank or isolates that were common to one more locations. Based on phylogenetic analysis, 97 isolates from three cities belonged to Phyla Proteobacteria, Actinobacteria, Firmicutes and bacterial group without matches or low affiliations in database (Fig. 2). Proteobacteria constitute the two clusters of the topmost clade encompassing most of the isolates from Quezon City and Mandaluyong City. Actinobacteria and Firmicutes comprised the clusters in middle clade. Branching out from the three phyla were clusters of bacterial groups with no matches or bacteria with generally low affiliations in the databank, which constituted bacterial isolates mostly from Marikina City.

DISCUSSION

Many consumers use filtered water from portable filtration systems or tap water directly from the faucet for drinking or other household consumption. However, there is limited information on the bacterial community composition in these environments. This present study elucidates the bacterial composition based on the molecular profiles of culturable bacteria isolated from biofilms and filtered water in portable filtration systems and tap water in three cities of Metro Manila. These bacteria were isolated using different growth media such as NA, TSA and PDA. The use of multiple non-selective media in isolation from environmental samples increases the opportunity to isolate more cultivable bacteria. This was shown by diverse bacterial composition and number in each culture medium. Many of the bacterial isolates grew in specific growth medium except for some isolates like Q-BN2 and Q-BP2 (both associated with *Pseudomonas* sp. Cf0-4) that grew in NA and PDA and MD-BN5 and MD-BT3 (both related to *Delftia* sp. C-5) that thrived in NA and TSA. PDA is a known medium specific for fungal culture and isolation. However, it has not been utilized for bacterial isolation from environments. PDA has been used in some studies to test bacterial inhibition of fungal growth, where bacterial isolates were only allowed to diffuse onto PDA culture plates (Molva & Baysal, 2014; Sivanantham et al., 2013; Khan et al., 2010). This present study is the first report on the use of PDA for isolation and culture of bacteria from water environment. Further studies are needed to investigate the extended use of PDA for bacterial culture and isolation from different environments including the mechanisms of bacterial growth in PDA. Despite using different growth media, still the number of bacteria isolated was relatively few (97 isolates). This could be expected because in freshwater

environments only 0.25 % of bacteria are culturable (Amann et al., 1995). Alternatively, pyrosequencing or metagenomics may be used in future studies for comprehensive identification of bacteria in biofilms and filtered water in portable filtration systems and tap water.

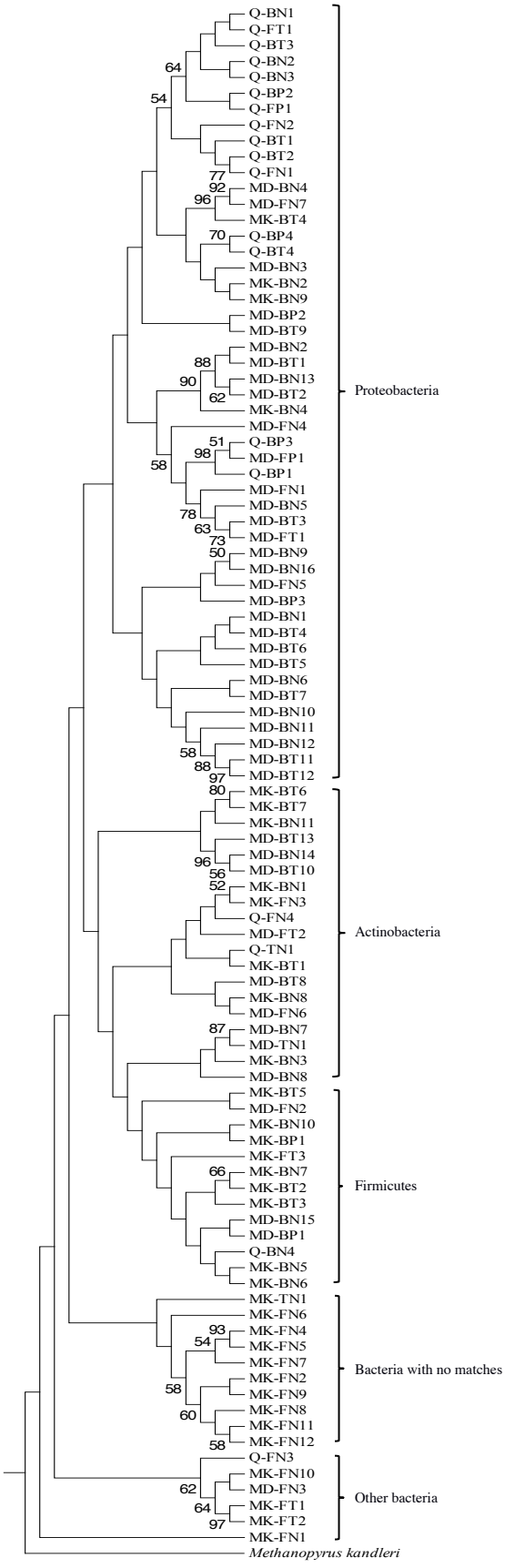

Figure 2. Phylogenetic relationship of bacterial isolates from biofilms, filtered water and tap water in three sampled cities. The phylogenetic tree was constructed using the Maximum-Likelihood method. Genetic distances were computed using Kimura 2-Parameter (K2+G) model. Numbers beside branches represent bootstrap scores above 50% from 1000 bootstrap iterations.

Based on 16S rRNA gene sequences of isolated bacteria, BLAST and phylogenetic analyses revealed more bacterial diversity in isolates obtained from Mandaluyong City than other cities. It was composed of isolates associated with different genera of β and γ Proteobacteria, Actinobacteria and Firmicutes. Besides, it also showed different strains of *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*. While isolates in Quezon City were predominated with Proteobacteria associates like *Pseudomonas*, 60% of the isolates in Marikina City were associated with uncultured bacterial clones and without affiliates in the data bank. This would suggest that these isolates have different identities or these are novel species or strains. It is also interesting to note that while most of the isolates were exclusive in specific city, some strains were found also in other cities. This is exemplified by *Cupriavidus* sp. TSA49, same associate of isolates Q-BP3 in Quezon City and MD-FP1 in Mandaluyong City and *Stenotrophomonas* sp. Hy3tC5, an affiliate of isolates MD-BT1 of Mandaluyong City and MK-BN4 of Marikina City. This might be expected because these three cities have a common source for drinking water. Absence of other isolates in across cities might be attributed to specificity, scarcity or less abundance of the strains in sampled location or difference in sampling dates.

There were differences in number and composition of isolates obtained from biofilm of ceramic filters, filtered water and tap water. Generally, more bacteria were isolated in biofilms than in filtered water in three cities; tap water consistently had the least. Although water treatment plants ensure removal of impurities from drinking water, many factors can adversely affect water quality on the way to its consumers. One is the introduction of bacteria to the distribution networks via mechanical failures such as breaks and leakages of the pipelines in the distribution systems (Momba et al., 2000). Another factor is the formation of biofilms which can colonize drinking water filters or water distribution pipes (Daschner et al., 1996). This could also occur in portable filtration systems since biofilms develop on all surfaces in contact with non-sterile water (Flemming, 2011). It was reported that 95% of the bacterial population in a drinking water system are located not on the water phase but on biofilms growing on the surface of the container or distributing pipe (Flemming et al., 2002).

In this study, it was observed that filtered water also contained bacteria but with different composition and lower number than that of the biofilms. It was evident that on the ceramic filters many bacterial species and strains were trapped like the different species of *Pseudomonas* in Quezon City and strains of *Pseudomonas aeruginosa* in Mandaluyong City. However, there was a reduction in number of different species or strains or loss of some species in filtered water. Nevertheless, this has implication on the imperative use of portable filtration systems in households to minimize or if not completely control the intake of impurities and opportunistic bacterial pathogens. On the other hand, some species or strains isolated on ceramic filter were also found in filtered water. This was apparent in Quezon City-isolated bacteria such as Q-BP2, which was found in ceramic filters and Q-FP1 in filtered water, which were both were associates of *Pseudomonas* sp. Cf0-4 and Q-BT1 from biofilm and Q-FT1 from filtered water, which were both affiliates of *Pseudomonas* sp. J4AJ. This was also observed in Mandaluyong City isolates like MD-BN4 in biofilm and MD-FN7 in filtered water, which were related to *Acinetobacter* sp. A17 and isolates MD-BT3 in biofilm and MD-FT1 in filtered water, both were affiliated with *Delftia* sp. C-5. This could be attributed to filter overflow or ceramic filters not cleaned due to excessive deposits of biofilms. Filter overflow increases the bacterial load of filtered water when water from the filter lip seeps onto the rim of the collection receptacle (Baumgartner et al., 2007). There is, therefore, a need to clean the ceramic filter regularly particularly when deposits of biofilms occur due to prolonged use to prevent or minimize contaminations in filtered water. Besides, the portable filtration system used in the study contained 0.9-μm ceramic filter, which may not completely trap the bacterial cells in ceramic filter cartridge. Thus, allowing some bacteria to thrive in water filter cartridge with granular activated carbon and silver. Although granular activated carbon in portable filtration system aids in disinfection, it does not guarantee the destruction of all bacteria (USEPA, 2001). Further studies are needed for different portable filtration systems available in the market in improving the quality of drinking water and their effects in human health. While ceramic filters and filtered water contained many bacteria, unexpectedly, only a single isolate was cultured in tap water for each city. This could be attributed to chlorination and disinfecting residues causing decline in bacterial phylotypes (Poitelon et al., 2010). Besides, continuous supply or flushing of water might have limited the growth of bacteria in tap water. Bacteria were detected less frequently and at lower concentrations in samples from taps supplied continuously compared to those supplied intermittently (Kumpel & Nelson, 2013).

Isolated bacteria from biofilms, filtered water and tap water in three sampled cities were related to different genera or species. Based on BLAST and phylogenetic analyses, these isolates were associated with bacteria found in natural water flora, opportunistic bacterial pathogens, soil bacteria or activated sludge bacteria. *Stenotrophomonas, Pseudomonas* and *Acinetobacter* are representative genera that are naturally found in potable water (Rusin et al., 1997). Byrd et al. (1991) pointed out that *Pseudomonas* spp. attained the highest survival in drinking water compared to other gram-negative bacteria. Silby et al. (2011) supported it with a comprehensive report on the metabolic flexibility of *Pseudomonas* spp. enabling them to adapt to various terrestrial and aquatic environments. Different strains of *Pseudomonas aeruginosa* (MD-BN6, MD-BN9, MD-BN10, MD-BN11, MD-BN12, MD-BT4, MD-BT5, MD-BT6, MD-BT7, MD-BT11 and MD-FN5), many of which were from soil , were isolated mostly from biofilms in Mandaluyong City. *P. aeruginosa* is a ubiquitous soil and waterborne opportunistic pathogen that constitutes a health risk to immunocompromised patients (Herath et al., 2014). In addition, it is regarded as an indicator of fecal contamination (Warburton, 1993; Clesceri et al., 1998). *Stenotrophomonas maltophilia* is an important nosocomial pathogen of immunocompromised patients (Safdar &

Rolston, 2007). Cases of *S. maltophilia* outbreaks due to defective sink drain and contaminated storage tanks for deionized water were previously reported (Verweij et al., 1998). So far, there were no bacterial isolates associated with water bacterial pathogens that cause serious problems like *Salmonella, Campylobacter, Shigella, Vibrio, Escherichia, Yersinia* and *Legionella*. From three sampled cities, there were other isolates found to be associated with soil bacteria. These include the following isolates with their corresponding associates: Q-FN4 (*Kocuria* sp. 97H2c), MK-BN1 (*Kocuria rhizophila*), MK-BN5 (*Staphylococcus arlettae*), MK-FN3 (*Barrientosiimonas humi*), MK-FN6 (*Pseudoxanthomonas japonensis*), MK-FN11 (Beta proteobacterium clone Lpl3-225), MD-BN5, MD-BT3 and MD-FT1 (*Delftia* sp. C-5), MD-BN10, MD-BN11, MD-BT4, MD-BT7 and MD-FN5 (*Pseudomonas aeruginosa*), MD-BN15 (*Bacillus pumilus*) and MD-FN6 (*Microbacterium oleivorans*). The presence of soil bacteria in biofilms and filtered water would suggest pipe leakage and entry of soil bacteria in distribution systems or water supply. There were also isolates associated with bacteria involved in activated sludge such as MK-BN2 (Bacterium clone B38), MD-BN4 (*Acinetobacter* sp. A17) and MD-BT13 (Pseudomonadaceae bacterium ALE_F7). These bacteria maybe involved in breaking down of organic matter into carbon dioxide, water, and other inorganic compounds. The bacterial composition found on ceramic filters of portable filtration system, either as a result of bacterial growth or trap on the filters, has implications to consumers. This may reflect the bacterial strains or species that would have been taken in when the consumers directly drink the tap water, that is, without the use of portable filtration systems.

Many of the isolates have relatively low percentage homology (73- 98%) with the associated bacteria. Although this study showed associations of isolates with different types of bacteria in drinking water environment, further investigations are needed to verify the roles and functions of the isolates. There were also many isolates with very low percentage homology with bacterial affiliates or without matches in the data bank. These would suggest different identities or novelty of isolated bacteria. Further studies are needed using molecular, biochemical and fatty acid profiling approaches to confirm their identities. There have been studies on microbial communities in filtration systems; however, these are restricted to large scale membrane filtration systems (Chen et al., 2004; Kwon et al., 2011). Other studies include microbiological contamination of drinking water in commercial household water filter systems but it was limited to bacterial counts (Daschner et al., 1996). Some were restricted to microbial community analysis but in potable drinking water (Pindi & Yadav, 2011). So far, this present study is the first report on molecular profiling of culturable bacterial community in drinking water portable filtration systems.

CONCLUSIONS

This study reports the molecular profile of culturable bacteria found in biofilms and filtered water from portable drinking water filtration systems and tap water. It showed differences in bacterial community compositions of the three sampled cities. Some of the genera identified were part of the normal water flora; however, some genera were associated with soil bacteria, opportunistic bacterial pathogens and activated sludge bacteria. The soil bacteria in filtration systems may be attributed to pipeline leakages, installation and maintenance in distribution systems or supplied water itself. The presence of opportunistic pathogens in drinking water would pose public health risks particularly for the vulnerable adults and children. Although the use portable filtration system has been emphasized, further studies are needed for the different portable filtration systems available in the market to investigate their efficiency in improving the quality of drinking water including the effects to the consumers. In this study, there were more isolates in the filter effluent than in the tap water. It could be possible that the prolonged use of water filters may degrade the water quality, by allowing biofilm to grow and the chlorine residual to dissipate. Future studies should focus on expanding the investigation of drinking water quality status in other areas or countries and on examining the roles and functions of the isolated bacteria.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest related to the study, and in the preparation and submission of the manuscript.

CONTRIBUTION OF INDIVIDUAL AUTHORS

Dr. Edward A. Barlaan conceptualized the study, designed the experiments and wrote the manuscript. Janina M. Guarte and Chyrene I. Moncada performed the experiments.

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