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Research Article

ISOLATION, CHARACTERIZATION AND IDENTIFICATION OF MICROBES FROM SHOWER HEAD IN UNIVERSITY OF GONDAR ATSAE TEWODROS CAMPUS, ETHIOPIA

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ABSTRACT

Shower heads are particularly susceptible to bacteria. They are dark, worm, damp environment-the perfect host for bacteria to grow. A biofilm accumulates inside shower heads are provides a healthy environment for bacteria to grow. Shower usage provides a source for repeated exposure to microbes through aerosolization and/or direct contact. The objective of this study is to isolate, characterize and identify microbes from the biofilm of showerhead. There is little knowledge of either their prevalence or the nature of other microorganisms that may be delivered during shower usage. This study was conducted to explore the composition of the microbial community that resides within the shower head and to give awareness for community to clean their shower heads periodically and taking baths instead of showers whenever possible. The samples were collected by sterile swab sticks from the selected shower heads and preserved in sterilized bottle with normal saline until they reached the laboratory, where the samples were stored in a refrigerator at 4 °C. We followed standard microbiological technique to isolate the microbes from shower heads. The numbers of isolated microbes on PCA were 4.43 log CFU/ml to 6.48 log CFU/ml and 4.51 log CFU/ml to7.48 log CFU/ml on MCA. The predominant microorganisms on shower heads biofilm taken from nine bathrooms included, Staphylococcus aereus, Staphylococcus epidermis, Pseudomonas aeruginosa, E. coli, Mycobacterium phlei, Micrococcus luteus, Serratia marcescen, Aspergillus flavin and Aspergillus niger. Water from an untreated shower head contain different opportunistic microbes and can cause different disease.

KEY WORDS: aerosolization, biofilms, opportunistic pathogen, shower head.

INTRODUCTION

A biofilm is an assemblage of microbial cells that is highly associated with a surface and enclosed in a matrix of primarily polysaccharide material. It represents a significant and incompletely understood mode of growth for bacteria Kokare (2009). Non cellular materials such as mineral crystals, corrosion particles, clay or silt particles, or blood components, depending on the environment in which the biofilm has developed, may also be found in the biofilm matrix Rodney

(2002). Biofilms have great significance for public health, because biofilm-associated microorganism exhibit dramatically decreased susceptibility to antimicrobial agents Rodney (2001). Shower usage provides a source for repeated exposure to microbes through aerosolization and/or direct contact (Zhou *et al.,* 2007). In nature, microorganisms exist primarily by attaching to and growing upon living and inanimate surfaces. These surfaces may take many forms, including those found in soil and aquatic systems, those on the spectrum of indwelling medical devices, and those of living tissues such as tooth enamel, heart valves, or the lung, and middle ear. The common feature of this attached growth state is that the cells develop a biofilm. Biofilm formation is a process whereby microorganisms irreversibly attach to and grow on a surface and produce extracellular polymers that facilitate attachment and matrix formation, resulting in an alteration in the phenotype of the organisms with respect to growth rate and gene transcription (Rodney, 2001). Microorganisms universally attach to surfaces and produce extracellular polysaccharides, resulting in the formation of a biofilm. Biofilms pose a serious problem for public health because of the increased resistance of biofilm-associated organisms to antimicrobial agents and the potential for these organisms to cause infections in patients with indwelling medical devices. An appreciation of the role of biofilms in infection should enhance the clinical decision-making process (Rodney, 2001). A biofilm accumulates inside shower heads are provides a healthy environment for bacteria to grow. The concentration can be 100 times higher in shower water than in the supply water. These bacteria are aerosolized when water begin to flow. The bacteria can be inhaled deep inside the lungs in this form. The shower heads may present significant potential exposure to aerosolized microbes, including documented opportunistic pathogens (Zhou *et al.,* 2007). A shower uses less water than a full immersion in a bath. Some shower heads can be adjusted to spray different patterns of water, such as massage, gentle spray, strong spray, and intermittent pulse or combination modes. Hard water may result in calcium and magnesium deposits clogging the head, reducing the flow and changing the spray pattern. For descaling, various acidic chemicals or brushes can be used or some heads have rubber-like jets that can be manually descaled. A homemade remedy is to immerse it in a solution of water and vinegar for a while, since the vinegar is able to dissolve lime scale (Shove *et al.,* 2004). Shower usage provides a source for repeated exposure to microbes through aerosolization and/or direct contact. The inside of a showerhead is a specific niche that is moist, warm, dark, and frequently replenished with low-level nutrient resources and seed organisms. Biofilms form on interior showerhead surfaces and potentially expose the user to a cohort of unknown, aerosolized microorganisms. Shower aerosol particles can be sufficiently small to carry bacteria deep into the air ways (Zhou *et al.,* 2007). Pulmonary disease and other health risks such as asthma, bronchitis, and hypersensitivity pneumonitis are associated with inhalation of both viable bacteria and in viable microorganisms or their components (Marras *et al.,* 2005). It has been hypothesized that the rise in pulmonary infections by nontuberculous mycobacteria (NTM) over recent decades is linked to increased use of showers rather than baths (Krause, 2000). Immune-compromised populations are on the rise; thus, identification of anthropogenic reservoirs of potential pathogens is of public health concern (Exner *et al.,* 2005). Despite implication as a potential source of disease, the microbial composition of the showerhead environment is poorly known. Many of these microbes are closely related to organisms common in water, but some microbes of potential public health concern are enriched to high levels by the showerhead environment. The environments we humans encounter daily are sources of exposure to diverse microbial communities, some of potential concern to human health. Showers are important interface for human interaction with microbes through inhalation of aerosols, and shower-head waters have been implicated in disease. There is little knowledge of either their prevalence or

the nature of other microorganisms that may be delivered during shower usage. In this study microbial constituents of the shower-head bio-films will be identified.

The shower head may expose individuals to microbes that colonize the inside of these shower heads. Although the types of microbes that live there differ depending on the water source and other external factors, some of these microbes may be opportunistic pathogens that have the potential of harming immune compromised individuals such as a person with ADIS, flu, cancer and also pregnant women and children. Therefore, this study will be conducted to explore the composition of the microbial community that resides within the shower head and to give awareness for community to clean their shower heads periodically and taking baths instead of showers whenever possible.

MATERIALS AND METHODS

Study area and design

The study was conducted in University of Gondar at Tewodros Campus. Laboratory based experiment was conducted from February 2017 to June 2017 isolate, characterize and identify microbes from the shower-head.

Sample collection

The shower-heads of the nine bathrooms in the Tewodros Campus were randomly selected for this study. The samples were collected by sterile swab sticks from the selected shower heads and preserved in sterilized bottle with normal saline until they reached the laboratory, where the samples were stored in a refrigerator at 4° C. The microbial study was conducted at University of Gondar, Department of Biology, in Microbiology Laboratory.

Sample preparation

The collected samples were mixed with normal saline and homogenized in a flask for five to ten minutes. After homogenization, 1 ml of each sample was transferred aseptically into 9 ml of distilled water. The homogenates were serially diluted (10^{-1} - 10^{-10}) and 0.1 ml aliquot of appropriate dilutions was spread-plated in duplicate on pre-solidified plates and incubated at appropriate temperature and time for counts of different microbial groups. The colonies were counted and then expressed as colony forming units per gram (CFU/ ml) (Berhane *et al*., 2015).

MICROBIAL ENUMERATION

Aerobic Mesophilic Count (AMC)

For AMC, 0.1 ml aliquot was spread Plated on Plate Count Agar (Oxoid) (ISO method 4833:1991). The plates were incubated at 30- 35° C for 48 hours.

***Salmonella and Shigalla* count**

A dilution of 0.1ml aliquot was spread plated onto Salmonella Shigalla Agar (SSA) by using the spread plate method, according to ISO method 6888:1983. All plates were incubated at 37° C for 48 hours. These cultures were further analysed by standard biochemical tests.

Microbial Analysis

After enumeration of aerobic mesophilic bacteria, 10- 15 colonies with distinct morphological differences such as colour, size and shape was randomly picked from countable plates and inoculated in to tubes containing about 5 ml Nutrient Broth (Oxoid). These were incubated at 30 – 32^0 C over-night. Cultures were purified by repeated plating and maintained on appropriate slants at 4^0 C. For identification we used selected biochemical tests such as catalase test, triple sugar iron agar test, urease test, citrate utilization test, indole test, methyl red test and motility test after checking the cell morphology of the pure culture Gram type.

Yeast, Moulds and Other Fungus Counts

From appropriate dilutions, 0.1 ml aliquot was spread-plated on pre-solidified surfaces of Potato Dextrose Agar supplemented with 0.1g Chloramphenicol and incubated at 25-28 ^{0}C for 5-7 days. A smooth (non-hairy) colony without extension at periphery (margin) was counted as yeasts.

RESULTS

Isolation of Bacteria

Biofilms were obtained by swab of interior surfaces of 9 showerheads from three buildings in Tewodros campus. Microbes were first grown on Plate Count Agar, McConk Agar, and SS Agar for 24 h and then the colonies were counted. Microbes grow on Plate Count Agar was considered as aerobic mesophilic bacteria. McConk Agar is selective and differential media used to grow only gram-negative bacteria. McConk Agar was interpreted with pink colour colony as lactose fermenters and yellow colour colony as non-lactose fermenters. *Shigella Salmonella* agar is a selective media used to grow *salmonella* and *shigella*. A colony with black spot at the centre was considered as *salmonella* and round colony without black spot were considered as *shigella.* A round colony with pink colour and small in size were considered as *E. coli* (Table 1).

Table 1: major microorganisms (log cfu/ml) on Plate Count Agar, McConk Agar, and SS Agar

Samples	MCA/CUF	PCA/CUF	SSA/CUF
T-25(1)	4.62	4.43	5.57
T-25(2)	5.46	5.52	6.53
T-25(3)	6.66	5.42	6.60
T-28(a)	6.72	5.51	6.76
T-28(b)	5.04	5.57	5.1
T-28(c)	4.51	4.58	5.53
T-23(x)	7.48	6.48	6.18
T-23(y)	5.43	5.43	6.70
T-23(z)	5.58	4.54	5.65

Key: Cuf/ml=Colony× Dilution Factor, MCA= McCook Agar**,** PCA= Plate Count Agar, SSA= Shigalla Salmonella Agar

Isolation and characterization of Moulds and Yeasts

A smooth (non-hairy) colony without extension at periphery (margin) was counted as yeasts. Hairy colonies with extension at periphery were counted as moulds and greenish colour was counted as *Aspergillus flavin*, blackish was considered as *Aspergillus niger* on the Potato Dextrose Agar.

Table 2: Isolation and characterization of moulds and yeasts

Samples	shape	Color	Hyphea	Transparency	Species
T-25(1)	Round	Blackish	filamentous	T	*Aspergillus flavin*
T-25(2)	Round	Whitish	filamentous	T	*Aspergillus flavin*
T-25(3)	Round	Greenish	filamentous	T	*Aspergillus flavin*
T-28(a)	Wrinkled	Whitish	filamentous	T	*Aspergillus niger*
T-28(b)	Round	Black	filamentous	O	*Aspergillus niger*
T-28(c)	Wrinkled	Greenish	filamentous	O	*Aspergillus flavin*
T-23(x)	Wrinkled	Whitish	filamentous	T	*Aspergillus flavin*

T-23(y)	Mucoid	Whitish	No hyphea	-	- yeast
T-23(z)	Wrinkled	Whitish	filamentous	T	*Aspergillus flavin*

Key: O= Opaque, T= Transparent

Morphological and biochemical characterization of bacteria

Various bacteria were identified on shower heads from residential areas, as presented in Table.2. *Staphylococcus epidermidis* and *Staphylococcus aurous, Pseudomonas aeruginosa*, *Escherichia coli, Micrococcusluteus*, *Mycobacterium spp* and *Serratiama rcescens* were identified.

Table 3: Morphological and biochemical characterization bacteria

Samp.	G.st	Shape	Ind	MR	Cit	Mot	TSI	Ure	Coa	Ca	Bacterial species
T25(1)	+	Cocci	-	-	-	-	-	+	-	+	*S. epidermis*
T25(2)	-	Cocci	-	-	+	-	-	+	+	+	*P. aeruginosa*
T25(3)	+	Cocci	-	+	+	+	+	+	-	+	*M. phlei*
T28(a)	+	Cocci	-	-	+	-	-	+	-	+	*S. aureus*
T28(b)	-	Rod	-	-	+	-	-	+	-	+	*S. marcescens*
T28(c)	-	Rod	-	-	+	-	-	+	-	+	*E. coli*
T23(x)	+	Cocci	-	-	+	+	+	+	-	+	*M. luteus*
T23(y)	+	Cocci	-	-	+	-	-	+	-	+	*S. aureus*
T23(z)	-	Rod	-	-	+	-	-	+	+	+	*P. aeruginosa*

KEY:-G.st=Gram Staining, Ind=Indole test, MR-VP=Methyl Red test, Cit=Citrate test, Mot=Motility test, TSI=Triple Sugar Iron,Ure=Urease test, Coa=Coagulase test, Cat=Catalase test

DISCUSSION

It is thought that shower heads are a possible pathogen habitat, as they accumulate deposits. Staphylococcus spp. is known to cause diseases such as bacteraemia, but it can cause infections on inimmunocompromised patients (Lehtola, et al., 2007). The majority of showerhead micro biota encountered in current study is composed of genus or species level relatedness groups that are commonly found in water and soil. The showerhead environment strongly enriches for microbes that are known to form biofilms in showerhead, including Mycobacterium spp., Staphylococcus spp., Pseudomonas spp. and others. Feazel et al., (2009) reported that the detection of significant loads of M. avium in many showerhead biofilms. And the identified microbe has a potential personal health problem. The reasons for the enrichment of Mycobacteria spp. on shower head biofilm are not exactly known but their wax surface may be particularly resistant to shear forces generated in shower operation. Furthermore, many species of biofilm-forming Mycobacteria are chlorine resistant, and thus potentially can be enriched by chlorine disinfection protocols used by many municipalities (Feazel et al., 2009). Kelleyet et al. (2004) isolated Methylobacterium and Sphingonomas spp. from showerhead biofilms. In the current study these bacteria are not detected. The predominant bacteria

found in current study were Staphylococcus aereus, Pseudomonas aeruginosa, E. coli, Mycobacterium spp., Micrococcus spp. The original source of these microbes could be man or animal faeces contacted with lakes, pools, and water supplies for the shower water of the university and it is a clear indicater of faecal contamination of the water and having serious sanitary problem. The isolation of E. coli is also positive indication of recent faecal contamination of the water. Since E. coli is recent indicator of fecal contamination, the site of contamination might be after the water treatment plant. Pseudomonas aeruginosa occurrence in drinking water is probably related to its ability to colonize biofilms in plumbing fixtures (i.e., faucets, showerheads, etc.) than its presence in the distribution system or treated drinking water. According to Jooij et al. (2013); Warburton et al. (2007) report Pseudomonas aeruginosa even can survive in deionized or distilled water. Hence, it may be found in low nutrient or oligotrophic environments, as well as in high nutrient environments such as in sewage and in the human body. P. aeruginosa can cause a wide range of infections, and is a leading cause of illness in immunocompromised individuals (Thomson et al., 2013). According to Joonjin, (2013) report these isolated microbes cause opportunistic infection in immune-compromised person. In addition to bacteria, the fungal group, Aspergillus, spp., and some yeast were isolated. Shigella and Salmonella were not isolated from PCA even though they were isolated on SS agar this may be on PCA commensals and normal flora bacteria became dominant and there could be high nutrition and habitat competition.

CONCLUSION

The predominant microorganisms on shower heads biofilm taken from nine bathrooms included, Staphylococcus aereus, Staphylococcus epidermis, Pseudomonas aeruginosa, E. coli, Mycobacterium phlei, Micrococcusluteus and Serratia marcescen. Some of the isolates like Mycobacterium spp., and Pseudomonas spp. are opportunistic and potential human pathogens. Water from an untreated shower head could contain more bacteria than which are found in the toilet. Shower heads are particularly susceptible to bacteria because they are dark, worm, damp environment-the perfect host for bacteria to grow and proliferate. Immune-compromised individuals including people with HIV/AIDS or chronic lung disease, who are undergoing treatment for cancer or organ transplant, or who smoke, drink heavily or abuse drugs, pregnant women and neonates may be at risk for serious illness from these shower head bacteria.

RECOMMENDATION

Here are some tips that help to reduce the risk:

1. Use a low-flow shower heads, which is less likely to produce aerosol
2. Bacteria are more likely to attach to plastics, so choose a metal shower heads or a plastic one that is been treated with silver or another antimicrobial.
3. Disinfect the shower heads with chlorine bleach regularly to remove many microbes
4. If people are at risk for illness due to a compromised immune system, consider taking a bath or attaching a hose instead of a shower head.

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