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Investigation of Microbiological Quality of Water from the Feed Source to the Terminal Application in the Healthcare Facility: A Case Study

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Abstract:
Water treatment and distribution systems are highly dynamic and versatile in terms of microbial populations. Rigorous control, maintenance and monitoring schemes should be followed to ensure delivery of water with high microbiological standards of safety from the feed sources to the final consumption points. Otherwise, consequences will be devastating to human health and possibly life itself. The current study aimed to investigate the microbiological quality in healthcare facility in urban districts region in African country using standard pharmacopeia microbiological techniques. A random samples of water from different points from feed chlorinated municipal water, water treatment plants, point-of-use and final purified water in a product with a total number of samples reaching 105 bottles of water. Isolation of microorganisms was performed using Nutrient Agar (NA), MacConkey Agar (MAC), Tryptic Soya Agar (TSA) and Muller Hinton Agar (MHA). Microbial identification of the isolates was performed biochemically. Microorganisms from seven samples were not recovered from recovery media. Two isolates from 27 specimens could not be identified using biochemical techniques and require identification using molecular methods. Eight of the final consumable products showed signs of microbial contamination with one of them could not be identified biochemically. One of the products was contaminated with two different bacteria: *Escherichia coli* and *Staphylococcus vitulinus*. Three more samples were contaminated with *E. coli* while *S. aureus* was found in one sample. Two samples were found contaminated with *Pentoea spp*. water stations were found to be contaminated with *E. coli* and *S. lentus* in tanks. While the reverse osmosis (RO) units were found to be contaminated with *Pseudomonas luteola, Enterococcus columbae, Streptococcus uberis, E. coli* and *S. lentus*. Water from municipal sources showed the presence of *Pseudomonas luteola, Serratia ficaria, Pentoea spp, E. coli, S. aureus* and *S. vitulinus*. The study indicated that water system control and monitoring require crucial improvements.

Keywords:
NA, MAC, TSA, MHA, API, VITEK2, Escherichia Coli, Staphylococcus Vitulinus, Serratia Ficaria, Pentoea Spp

1. Introduction

Ensuring microbiological quality of water in the healthcare industry is a pivotal point because water is a key ingredient in many processing steps till the final consumption [1]. If the microbiological cleanliness of water is impacted at any point through its stream from the feeding source to the terminal use point or product, the threat to the health or even the life of the consumers may be serious with devastating safety and financial consequences [2]. A stress on appropriate maintenance management system, monitoring tools and frequency of testing is necessary because of the rapidly changing and dynamic nature of the bacterial community within a certain system which may flip from quality stable and safe system into chaotic and catastrophic one very swiftly.

Microbiological studies have been conducted previously on water systems in healthcare facilities that showed great diversity in bacterial populations that may include objectionable microorganisms [3]. These studies covered different processing stages of water starting from city water to the final purified water distributed from the loop to the use points [3].

An investigational study has been performed in one healthcare facility in urban districts region in an African country on the quality of its water system starting from the municipal water inlet source till the final dispensing into units for application by consumers. Quantitative and qualitative screening of random samples collected from water system should elucidate defects in the maintenance, control and monitoring systems, in addition to the degree of safety of water microbiologically.

2. Materials and Methods

2.1. Study Subject

Different fresh samples of water were sampled and analyzed during the same day without delay in the same day of the sampling from the healthcare firm comprises water treatment plants that convert chlorinated city water into purified water for distribution, use and consumption.

2.2. Water Sample Transfer Reservoir

The overall water sampling procedure was done under careful aseptic conditions. Water samples were collected in sterile autoclavable glass sampling containers with leak-proof screw cover. During sample collection, sufficient air headspace should be left to allow for mixing before testing. Samples representative of the water being tested were collected from each sampling port of waterline [4].

2.3. Water Sampling Process and Handling

Water effluents that contain Chlorine should be neutralized with Disodium Thiosulfate (one ml of a 10% solution for each liter of chlorinated water) prior to testing in the sampling bottle. Sampling points-of-use should be flushed and sanitized before conducting aseptic sampling. A detailed method for sampling without contamination has been described previously by guidelines such as ASTM and some researchers. The samples should be kept at 1-4 ℃ unless the transfer to the analysis laboratory is short [4-7].

2.4. Water Analysis in the Laboratory for Total Microbial Count

Microbiological water testing was conducted using standard microbiological methods using filtration technique as in pharmacopeia guiding principle as shown in Figure 1 [4-6, 8]. A random points-of-use and product samples were taken to cover different locations from the healthcare facility with a total number of 105 samples.

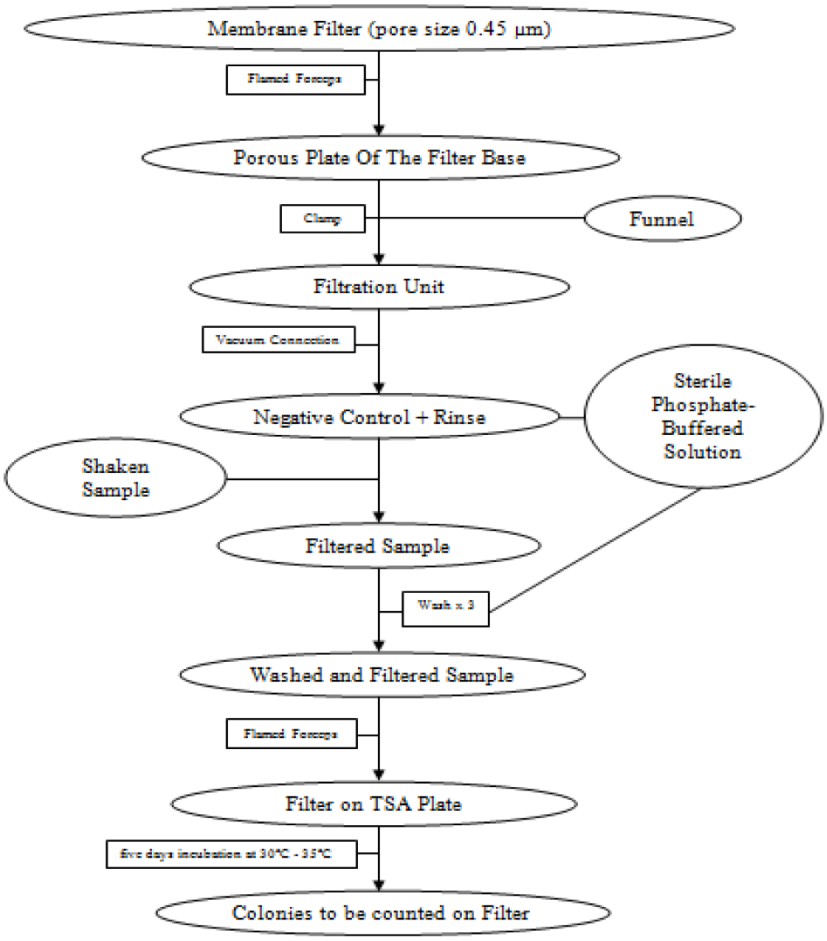

Figure 1. *Processing Steps for each water sample till incubation and counting on Tryptone Soya Agar (TSA) plates.*

2.5. Water Analysis in the Laboratory for Detection of Objectionable and Pathogenic Microbes

The same previous scheme was followed as in point (2.4) but Tryptone Soya Agar (TSA) plates were replaced with Pseudomonas Agar (PA), Eosin Methylene Blue (EMB) and Burkholderia Agar (BA) [4-6].

2.6. Microbial Preservation and Identification

Isolation of microorganisms for identification was performed using Nutrient Agar (NA), MacConkey Agar (MAC), TSA and Muller Hinton Agar (MHA). Microbial identification of the isolates was performed biochemically using API identification (ID) system and confirmed using VITEK2 compact system v 08.01 [9]. ID was performed as guided by the manual of the suppliers. Moreover, colonies isolated from the samples were preserved in cryogenic media and refrigerated using standard protocol followed in microbiology [10].

2.7. Quality Control of Culture Media

To verify the validity of the culture media used, growth promotion (GP) testing was performed using standard strains purchased from ATCC (American Type of Culture Collection, Manassas, Virginia) and processed as in standard procedure. All the nutrient media and chemicals were purchased from OXOID (Basingstoke, Hampshire) and Sigma-Aldrich (St. Louis, MO 63103), respectively. Standardized stable suspensions of test strains were handled as described in Seed-lot culture techniques (seed-lot systems) so that the microbial cells used for inoculation were not more than five passages from the source master seed-lot. All organisms were stored at -80 ℃ in a validated -86C Ultra-low temperature freezer (-86 Degree ULT Freezers, Qingdao Shandong, China) in a validated cryogenic environment, and reactivated only prior to study conduction. All media were sterilized by autoclaving in a validated autoclave (FEDEGARI FOB3, Fedegari Autoclavi SpA, SS 235 km 8, 27010 Albuzzano (PV), Italy). Microbial suspensions were determined by applying serial dilutions and plate counts using conditions and media suitable for each microorganism to select dilutions of concentration 300-1000 CFU/ml as a working suspensions. Microbial test suspensions were used as soon as the results of serial dilutions could be determined [10].

3. Results and Discussion

The summary of microbiological analysis is shown in Table 1. All culture media that has been used in the investigation of water system passed GP tests. No pathogenic or objectionable microbes were detected using selective culture media in the whole water samples. Among the total number of sampled water, only 34 (approximately 32 %) samples showed signs of microbial growth and the microbial counts were within the acceptance criterion (i.e. the colony forming units (CFU) were significantly below 100 CFU/ml). Strangely, microorganisms from seven samples could not be recovered again in the isolation and recovery media which constitute about the fifth from the positive specimens.Limitations like this have been discussed previously by some researchers [11]. Two isolates from 27 specimens (about 7 %) could not be identified using biochemical techniques and require further study using molecular methods (ex. Polymerase Chain Reaction (PCR)). Limitations of the biochemical identification system and the advantages of the new molecular technologies have been reported earlier by some researchers [12, 13]. Eight (approximately forth of total positive samples) of the final consumable products showed signs of microbial contamination with one of them could not be identified biochemically. One of the products was contaminated with two different bacteria: *Escherichia coli* and *Staphylococcus vitulinus*. Three more samples were contaminated with *E. coli* while *S. aureus* was found in one sample. Two samples were found contaminated with *Pentoea spp* (a member of the family Enterobacteriaceae) [14].

Table 1. *Summary of microbiological analysis of water system.*

Microbiological Media	GP Test[1]	Enumeration	Pathogen Detection
TSA	Pass	71 samples 0 CFU/100 ml, 34 samples <10 CFU/ 100 ml	N/A
BA	Pass	N/A	No
PA	Pass	N/A	No
EMB	Pass	N/A	No

[1] Growth Promotion (GP) test was done according to USP N/A: Not Applicable

On the other hand, water treatment stations were found to be contaminated with *E. coli* and *S. lentus* in tanks. While the reverse osmosis (RO) units were found to be contaminated with *Pseudomonas luteola, Enterococcus columbae, Streptococcus uberis, E. coli* and *S. lentus*. Water from municipal sources showed the presence of *Pseudomonas luteola, Serratia ficaria, Pentoea spp* (both are members of the family Enterobacteriaceae), *E. coli, S. aureus* and *S. vitulinus*. Different authors and researchers have demonstrated similar findings in water system [15-23]. On the other hand, the diversity of microbial population in water in similar situations has been demonstrated in other work related to pharmaceutical field [24]. Microbial distribution in water samples is illustrated in Figure 2. The percentage of samples that could not be recovered or identified is shown in Table 2. Prevalence of Gram-negative over Gram-positive bacteria in water has been demonstrated before in previous research with several bacterial families or genera are in common [3].

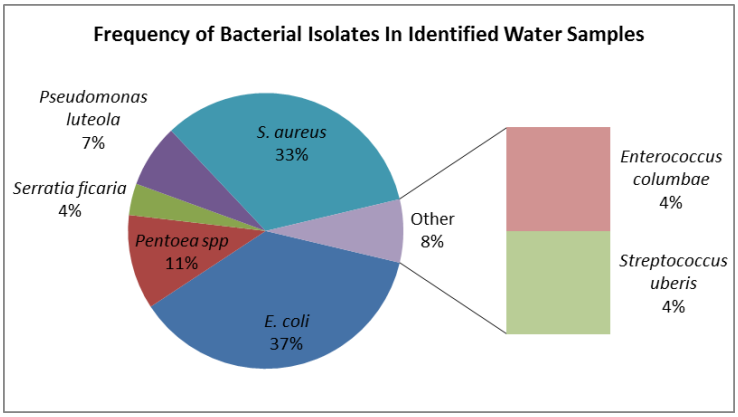

Figure 2. *Rate of bacterial isolates detection in identified water samples.*

The demonstrated limitations of the culture-based media (recovery problem and skipping pathogen detection) may indicate the need for the implementation of more reliable supportive techniques such as molecular techniques [25-27]. Replacement of the conventional microbiological techniques with new rapid microbiological methods (RMMs) - especially those technologies which do not rely on culture-based methodologies - will significantly improve sensitivity and accuracy of detection, enumeration and identification of microbial cells within much shorter time.

The current study showed the resilient nature of Enterobacteriaceae to distribute in water (notably *E. coli*) being easily spotted and located at several sections, from the original firm supply of city water to the final point-of-use. Using water treatment stations are not an absolute guarantee for the microbiological safety of the product water. Correct maintenance of the water treatment plant and the quality of the feed water supply are also a key contributing factor. The present study showed that the barriers of the water plants could be surpassed by creeping microbes and many of them are pathogenic and may impose threatening to the life of the final consumers [26, 27].Such threat from Enterobacteriaceae family members should not be underestimated as they constitute with *S. aureus* a globally known health problem issue [28-30].

A growing list of the objectionable microorganisms coupled with increasing number of ill populations - especially those with problems in the immunity system - among mankind may sound a warning alarm for a great risk of possible future devastating global outbreaks that may storm the civilization.

Table 2. *Microbial distribution in water samples from the total number of samples.*

	From Total Samples	Gram Positive	Gram Negative
Identified Positive Samples	26%	41%	59%
Unidentified and Unrecovered Positive Samples From Total Water Samples	9%	**Unrecovered Positive Samples** 7%	**Unidentified Positive Samples** 2%
Unidentified and Unrecovered Positive Samples From Total Positive Samples	26%	21%	6%

4. Conclusions

A highly dynamic and versatile nature of water system is a challenging task that requires rigorous monitoring, maintenance and control of the system using advanced technologies to ensure delivery of water/product with appropriately safe microbiological quality to the final user. The present case showed that the existing water system needs strict maintenance, sanitization and control for the microbiological quality of the feeding source of water as the produced water is not safe for human consumption. Moreover, implementation of RMM in the monitoring of water microbial quality should be included in an extension long-term study for the current investigation to compare both conventional and new microbiological techniques and elucidate the gap between them to determine the necessity and the degree of the replacement for the old microbiological methods with the new technologies.

Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this article.

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