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Microbiological Analysis of Water Used for Pharmaceutical Product Preparation

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Abstract:
Water is necessary for manufacturing, medicinal and clinic reasons, in the formulation and processing of medications and additional well-being merchandises and for clean-up and sanitation reasons. Water is most important and widely used in the pharmaceutical industry as a natural resource ingredient, and solvent in the dispensation, active pharmaceutical ingredient preparation, and production of medicinal products and intermediates. Regulator of the excellence of water all through the manufacture, storages and delivery processes, as well as bacteriological and organic quality, is a most important concern. Therefore, Present study employed to determine the microbiological prevalence within the waters used for pharmaceutical product preparation. An experimental and prevalence study was employed. Sixteen samples were collected from a reputed pharmaceutical company located at Dhaka, Bangladesh. Water sampled from the production area of different medicine. Conventional cultural method and biochemical methods were carried out to enumeration of total microbial load and isolate the specific pathogens from the collected water samples. The result found that, the total aerobic viable bacterial load in all samples tested was found to be within the prescribed limit (<100 cfu/ml). Out of 16 samples, 3 samples were found to be proliferated with Pseudomonas spp. Our study found that only one sample (sample no 11) was found to be proliferated with Escherichia coli. On the other hand no evidence of Staphylococcus aureus was found in any sample. As well as Salmonella spp. was absent in all sample. Overall, the current investigation clearly imparts a complete bacteriological profile of pharmaceutical water and reveals the presence of specific pathogens may lead to the serious toxicity and hazard of the finished pharmaceutical products.
Keywords*: Water, Microbiological profile, Pharmaceutical water, Product preparation, Pseudomonas spp.*

I. Introduction

Water is the one of the major commodities used by the pharmaceutical industry. It is widely used as a raw material, ingredient [1], and solvent in the processing, formulation, and manufacture of pharmaceutical products, active pharmaceutical ingredients (APIs) and intermediates, and analytical reagent [2]. It may also present as an excipient, or used for reconstitution of products, during synthesis, during production of finished product, or as a cleaning agent for rinsing vessels, equipment and primary packing materials etc. There are many different grades of water used for pharmaceutical purposes [3]. Several are described in USP monographs that specify uses, acceptable methods of preparation, and quality attributes [4].

Water is the most widely used substance, raw material or starting material in the production, processing and formulation of pharmaceutical products. It has unique chemical properties due to its polarity and hydrogen bonds. This means it is able to dissolve, absorb, adsorb or suspend many different compounds [5]. These include contaminants that may represent hazards in themselves or that may be able to react with intended product substances, resulting in hazards to health. Different grades of water quality are required depending on the uses and route of administration of the pharmaceutical products.

Control of the quality of water throughout the production, storage and distribution processes, including microbiological and chemical quality, is a major concern. Unlike other product and process ingredients, water is

usually drawn from a system on demand and is not subject to testing and batch or lot release before use. Assurance of quality to meet the on demand expectation is, therefore, essential [6]. Additionally, certain microbiological tests may require periods of incubation and, therefore, the results are likely to lag behind the water use. Some types of microorganism may proliferate in water treatment components and in the storage and distribution systems. It is very important to minimize microbial contamination by routine sanitization and taking appropriate measures to prevent microbial proliferation. Pharmaceutical water production, storage and distribution systems should be designed, installed, commissioned, validated and maintained to ensure the reliable production of water of an appropriate quality. These systems should not be operated beyond their designed capacity. Water should be produced, stored and distributed in a manner that prevents unacceptable microbial, chemical or physical contamination [7].

Due to its criticality in pharmaceutical production, microbiological control of water is of great importance. Because water is ever present, each grade of pharmaceutical water is a potential source of microbiological contamination, especially when not properly controlled [8]. Control is not only about numbers of microorganisms recovered through bio-burden testing for microbiologists additionally need to understand the types of organisms present within water [9]. This is in order to look for changes to trends and to understand if they are indicators of more serious problems (like bio-films) or if they present a special risk to products (and thus to patients).

Even though most pharmaceutical water systems are controlled, microorganisms will sometimes be present in low numbers. The need for microbiologists to consider the constant and changing patterns of microorganisms found in water systems are varied [10].

Control of the microbiological quality of pharmaceutical water is a high priority. Some types of microorganism may proliferate in water treatment components and in the storage and distribution systems. It is crucial to minimize microbial contamination by proper design of the system, periodic sanitization and by taking appropriate measures to prevent microbial proliferation.

Water used for pharmaceutical purposes can be divided into two general types: bulk waters, which are typically produced on site where they are used; and packaged waters, which are produced, packaged, and sterilized to preserve microbial quality throughout their packaged shelf life. There are several specialized types of packaged waters, differing in their designated applications, packaging limitations, and other quality attributes. Purified Water is used as an excipient in the production of nonparenteral preparations and in other pharmaceutical applications, such as cleaning of certain equipment and nonparenteral product-contact components. Unless otherwise specified, Purified Water is also to be used for all tests and assays.

Many industries suffer from the microbial contamination of ultrapure water (UPW). These include the pharmaceutical, food, and beverage industries. The presence of even a single bacterial cell and/or the products of cellular degradation, can severely compromise the quality of the final product [11, 12]. Therefore, appropriate maintenance of pharmaceutical water management system is essential to ensure the final product safety and non-toxicity [13]. Indeed in Bangladesh, microbiological contamination of various waters is very common [14]. Besides, the pharmaceutical products have been found to be contaminated with an array of microorganisms; however, the water used for the manufacturing purpose has not been analyzed yet [15].

There are few published references as to the expected micro flora in processed water and even fewer that pertain to the pharmaceutical industry. The paucity of necessary information in this respect, the present study attempted to determine the microbiological prevalence within the waters used for pharmaceutical product preparation and to isolate the pathogenic organisms such as *Staphylococcus aureus, E.coli Salmonella spp and P. aeruginisa etc* from the collected purified water samples by using standard tests.

II. Material And Methods

Study Design: It was an experimental and prevalence study

Study Duration: November 2018 to April 2019

Place of study: This study was conducted at central microbiology lab of Department of Microbiology, Stamford University of Bangladesh, Dhaka, Bangladesh.

Sample size: 16 samples were collected

Sample collection: Sixteen samples were collected from different area in purified water system. Water was collected from the reservoir of a pharmaceutical company at Dhaka, an industrial city of Bangladesh. Water sampled from the production of different medicine was collected directly into sterile plastic container and put on ice and immediately taken to the laboratory for routine microbiological and other analyses. Storage was at 4°C prior to analysis and assays.

Microbial analysis:

Enumeration of total microbial load: Enumeration of total microbial load was performed by the membrane filtration method [16]. After completion of filtration of water the filter paper was picked up aseptically from filter unit, and was placed onto the R2A agar media following incubation for 5 days at 30-35ºC.

Test for the presence of specific pathogens:

Staphylococcus aureus: Presence of specific pathogens was detected by using different selective media. Filter 100 ml of sample through 0.45 µ membrane filter. Transfer membrane filter to 100 ml sterile soybean casein digest medium and include at 30-35 °C for 24 hours. For the detection of the presence of *Staphylococcus aureus*, the filter paper was placed onto mannitol salt agar (MSA) plate and incubated at 30–35 °C for 18–72 h. Appearance of the yellowish white colonies surrounded by a yellow zone was indicative of *S. aureus.*

***Escherichia coli*:** Filter 100 ml of sample through 0.45 µ membrane filter. Transfer membrane filter to 100 ml sterile soybean casein digest medium and include at 30-35 °C for 24 hours. For the detection of *Escherichia coli*, streak a portion of enriched soybean casein digest medium on the surface of sterile MacCpnkey agar medium. Incubate the plates at 30-35 °C for 18-72 hours. After incubation presence of brick red colonies on MacConkey agar indicate the presence of *Escherichia coli*. Carry out further confirmation by streaking the colonies on the surface of Levine eosine methylene blue agar medium. Blue black colonies with metallic sheen confirm the presence of *Escherichia coli*.

Pseudomonas aeruginosa: Streak a portion of the enriched soybean casein digest medium on the surface of cetrimide agar medium and incubate at 30-35 °C for 18-72 hours. A greenish colored colony indicates the possibility of presence of *Pseudomonas aeruginosa*. Carry out further confirmation by pigment and oxidase tests. Streak representative suspect colonies from the surface of cetrimide agar on to the surface of pseudomonas agar medium for detection of fluorescein and pseudomonas agar medium for detection of pyocyanin. Cover and invert the inoculated plate and incubate at 30-35 °C for not less than 3 days. Examine the streaked surface under ultra-violet light. Examine the plates to determine colonies. If growth of suspect colonies occurs place 2 or 3 drops of a freshly prepared 1% w/v solution of tetramethyl-4-phenylenediamine dihydrochloride on filter paper and smear with the colony; if there is no development of a pink color, changing to purple, the sample meets the requirements of the test for the absence of *Pseudomonas aeruginosa.*

Salmonella* spp*: Filter 100 ml of sample through 0.45 µ membrane filter. Transfer membrane filter to 100 ml sterile soybean casein digest medium and include at 30-35 °C for 24 hours. For the detection of *Salmonella spp.* transfer 0.1 ml of enriched soybean casein digest medium to 10 ml of Rappaport Vassiliadis Salmonella enrichment broth and incubate at 30-35 °C for 24-48 hours. Streak above media on the surface of Wilson and Blairs BBS agar plate and incubate at 30-35 °C for 24-48 hours. Growth of green colonies with the blank center and in 48 hours the colonies become uniformly black. Colonies surrounded by a dark zone and metallic sheen indicate the possibility of the presence of *Salmonella*. If sub-cultured on a xylose-lysine-deoxychoolate and incubate on 30-35 °C for 24-48 hours. Well developed red colonies with or without black center indicate the possibility of *Salmonella.* Carry out further confirmation by streaking the colonies on the surface of triple sugar-iron agar by first inoculating the surface of the slant and then making a stab culture with the same inoculating needle and at the same time inoculate a tube of urea broth. Incubate at 30-35 °C for 18 to 24 hours. The formation of acid and gas in the stab culture and the absence of acidity from the surface growth in the triple sugar iron agar, together with the absence of a red color in the urea broth, indicate the presence of *Salmonella.*

III. Results and Discussion

Pharmaceutical manufacturing, like medicine, drug and injection fluid depends on water. The design, operation and maintenance of pharmaceutical-grade water systems are critical, both to keeping drug manufacturing facilities running and to ensuring final product quality [17]. Unfortunately, improperly maintained and operated systems rank very high on the list of problems cited during FDA and other regulatory inspections. Maintenance and operation are not "set and forget" activities [17, 18]. They require process adjustments and system maintenance. Indeed, pharmaceutical water systems need to be operated and maintained in a controlled manner that requires that the system be validated to provide assurance of operational stability and that its microbial attributes be quantitatively monitored against established alert and action levels that would provide an early indication of system control.

Table no 1: Microbial load in the water samples tested

Sample No.	Total aerobic microbial count (CFU/ml)	*Escherichia coli*	*Staphylococcus aureus*	*Salmonella* spp.	*P. aeruginosa*
1	12	-	-	-	-
2	07	-	-	-	-
3	04	-	-	-	-
4	05	-	-	-	-
5	08	-	-	-	-
6	17	-	-	-	+
7	06	-	-	-	-

8	32	-	-	-	+
9	14	-	-	-	-
10	12	-	-	-	+
11	17	+	-	-	-
12	20	-	-	-	-
13	22	-	-	-	-
14	18	-	-	-	-
15	14	-	-	-	-
16	25	-	-	-	-

Note: +, indicate Presence of bacteria; -, indicate absence of bacteria

In the present study, the total aerobic viable bacterial load in all samples tested was found to be within the prescribed limit (i.e., <100 cfu/ml). Out of 16 samples, 3 samples include sample no 6, sample no 8 and sample no 10 were found to be proliferated with *Pseudomonas* spp. as shown in Table 1. Our study found that only one sample (sample no 11) was found to be proliferated with *Escherichia coli.* On the other hand our study found that no evidence of *Staphylococcus aureus* found in any sample. As well as *Salmonella spp.* was absent in all sample. Even the total bacterial load was within the limit, the presence of specific pathogens may lead to the serious toxicity of the finished pharmaceutical products [15].

IV. Conclusion

In conclusion, we conclude that, the total aerobic viable bacterial load in all samples of water used in pharmaceutical industry for manufacture of medicine and drugs was found to be within the prescribed limit (i.e., <100 cfu/ml). As the pharmaceutical industries are growing to their excellence in Bangladesh, not only the raw materials for manufacturing, but also the microbiological traits of the waters should be considered for the sake of the finished product safety, which in turn, would be effective in public health management. Routine monitoring of pharmaceutical water is thus recommended.

References

[1]. Mehta G, Prabhu SM, Kantawala D. Industrial wastewater treatment-The Indian experience. J. Indian. Assoc. Environ. Management. 1995. 22, 276-287 .

[2]. Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT. Pharmaceuticals, hormones and other organic wastewater contaminants in U.S. Environ. Sci. Technol. 2002.36, pp 1202-1211.

[3]. Stmzeski EJ. 1980. Status of wastes handling and waste treatment across the pharmaceutical industry and effluent limitations. Proceedings of the 35th lndustrial Waste Conference, Purdue University, West Lafayette, IN. p. 1095-1108.

[4]. Seif HAA, Joshi SG, Gupta SK.. Effect of organic load and reactor height on the performance of anaerobic mesophilic .and thermophilic fixed film reactors in the treatment of pharmaceutical wastewater. Environ. Technol. 1992. 13, l16l-1168.

[5]. Andersen DR.. Pharmaceutical wastewater treatment: A case study. Proceedings of the 35s lndustrial Waste Conference, Purdue University, West Lafayette. IN, 1980. p.456462.

[6]. Struzeski EJ. Waste Treatment and Disposal Methods for the Pharmaceutical Industry. NEIC- Report EPA 1975.330/1-75-001; U.S. Environmental Protection Agency. Ofice of Enforcement, National Field Investigation Center: Denver, CO'

[7]. Molof AH, Zaleiko N.. Parameter of disposal of waste from pharmaceutical industry. Ann. N.Y. Acad. Sci. 1965. 130, 851-857.

[8]. Geldreich EE, Taylor RH, Blannon JC, Reasoner DJ. Bacterial colonization of point-of-use water treatment devices. J Am Water Works Assoc 1985;77(2):72-80.

[9]. Sandle T. An approach for the reporting of microbiological results from water systems. PDA J Pharm Sci Technol. 2004;58(4):231–7.

[10]. Jiminez, L. Microorganisms in the Environment and their relevance to Pharmaceutical Processes. In; Jiminez, L, editor. Microbial Contamination Control in the Pharmaceutical Industry. New York: Taylor & Francis; 2004. p. 8-9.

[11]. Tsuchizaki, M.. Measurement of microbes and particulates in ultrapure water and chemicals for the electronic industry. Semiconductor World 1983: 1–20.

[12]. Kulakov LA, McAlister MB, Ogden KL, Larkin MJ, O'Hanlon JF. Analysis of bacteria contaminating ultrapure water in industrial systems. *Appl Environ Microbiol*. 2002;68(4):1548-1555.

[13]. WHO (World Health Organization). 2003. *Assessing microbial safety of drinking water- improving approaches and methods*. WHO & OECD, IWA publishing, London, UK.
[14]. Acharjee, M., Jahan, F., Rahman, F. and Noor, R.. Bacterial proliferation in municipal water supplied in Mirpur locality of Dhaka city, Bangladesh. *Clean – Soil , Air, Water*. 2013. DOI: 10.1002./clen.201200618
[15]. Khanom. S., Das. K. K., Banik. S., Noor R. Microbiological analysis of liquid oral drugs available in Bangladesh. Int J Pharm Pharm Sci, 2014. Vol 5, Issue 4, 479-482
[16]. Cappuccino, J. G. and Sherman, N. 1996. *Microbiology- A Laboratory Manual*. The Benjamin/ Cummings publishing Co., Inc., Menlo Park, California.
[17]. Ramyasree M., Gangadharappa H. V., Deepak A. M. Review article water for pharmaceutical use. World Journal of Pharmaceutical Research.2018, 7 (10) 147-160
[18]. FDA 1993. Guide to inspections of high purity water systems Maryland, US Food and Drug Administration.