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Microbial pollution challenges for the Pharmaceutical Plants in Sikkim, India

Asha Subba[1], Manoranjan Pandey[1], Karma G. Dolma[2], Sangeeta Jha[1] and Ajeya Jha[1]

[1]SMIT, (Sikkim Manipal University), Tadong, Sikkim 737 132, India
[2]SMIMS (Sikkim Manipal University), Tadong, Sikkim 737 132, India

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ABSTRACT

Quality is a predominant theme in pharmaceutical production. As the most important raw material used in pharmaceutical industry, water quality remains central to the efforts to build quality in final products. Sikkim, the latest hub of pharmaceutical industries, has water streams the main source of water for them. This study had been undertaken to ascertain the magnitude of pathogenic microbial pollution to which Pharmaceutical industries in Sikkim are exposed to, and the challenges this poses to their water purification process. Our conclusions in this respect are that presence of pathogenic microbes in feed water to pharmaceutical country is extremely high. While ranking the water purification process steps using risk priority number, determined on the basis of expert opinion of quality assurance managers. Missing of Sodium Hypochlorite dosing emerged as the most critical step in this respect, followed by Pipe induced microbial growth and malfunctioning Reverse Osmosis (RO) membrane malfunctioning. Implications for the quality professionals and regulatory authorities are that the extent of microbial pollution is extremely high and that some steps in purification process are highly critical and validation process should take this into consideration.

Key words : *Pathogenic microbial pollution, Sikkim, Pharmaceutical industry, Water purification,*

Introduction

The "quality' in the pharmaceutical industry has emerged as an important principle. To synchronize its policies and practices an introduction of current good manufacturing practices has been crystalized and there has been a mounting emphasis on the importance of the quality of the pharmaceutical products (Woodcock 2004). To achieve this Substantial amounts of industrial and federal resources are invested in achieving quality (Bruttin and Dean, 1999). Water is one of the most widely raw material used in the pharmaceutical industry (WHO, 2006). Water also is the main priority to ensure high quality of pharmaceutical products (Pahwa *et al*., 2010; Rimantho, 2017).

Water is susceptible to microbial pollution involving innumerable pathogens—bacteria, viruses, protozoa, and helminths. According to the WHO, 80% of all diseases in the developing countries results from contaminated water (Kemprai *et al*., 2021). Contamination of surface water can be traced to animal (including human beings) waste in fields and feedlots beside waterways, meat packing and tanning plants and a few wildlife species, which transmit waterborne diseases. The spectrum of pathogenic and potentially pathogenic micro-organisms spread by water is extensive. The most frequent are the causative agents of intestinal diseases (typhoid, paratyphoid, salmonellosis, tuberculosis, brucellosis, tularaemia, leptospirosis, cholera, amoebic dysentery, schisostomiasis) (Sasakova, *et al*., 2018)

One of the primary objective of the microbiological control of pharmaceutical raw materials is to exclude possibility of any microbial content that may lead to deterioration of the product or may harm the patient (Russel, 2017). Microbial growth is a major challenge in pharmaceutical industry as it can result in a noticeable deficiency of water quality (e.g. taste, scent, turbidity, staining) and additionally inacceptable microbes multiplication (Bartram *et al.,* 2004; Boe-Hansen *et al*., 2002; Hammes *et al*., 2008; Juhna *et al*., 2007; Vital *et a*l., 2010; Vital *et al*., 2008,).

Water purification process comprises of following broad steps:

a. Filtration and chlorination of water
b. Water softening to
c. Activated carbon to remove chlorine and other organic materials
d. Filtration
e. Ion Exchange
f. Reverse osmosis to remove a large portion of salt and particulates, bacteria, and pyrogenic materials
g. Distillation
h. Ultraviolet radiation
i. The heating and storing of water

Sikkim is a Himalayan state located in eastern Himalayas (Chettri, *et al*., 2020) sandwiched between Bhutan in the east, Nepal in the west, Tibet in North and state if west Bengal in the South. It is a state renowned for its biodiversity (Jha *et al*., 2020) and unpatrolled natural beauty (Jha, *et al*., 2017). Being ecologically fragile (Mishra, *et al*., (2013), it has several limitations while selecting economic sectors for growth. As a policy Sikkim focusses on low volume - high value products (Mishra, 2014). This tiny state now harbors over 40 pharma companies. Till 2014-15, an investment of $414.73 million had been made and the figures continue to grow. With this recent surge of pharmaceutical companies, the state is in the league of Gujarat, Maharashtra and Himachal Pradesh in being a hub for the pharma industry (Das, 2019).

In Sikkim, Pharmaceutical companies are dependent on supply of water mainly from the numerous streams also known locally as *jaldhara*. In general, surface water is more susceptible to contamination from human sources (Winter *et al.,* 1998). This is unlike the pharmaceutical hubs in other parts of India, where water is mainly sourced from deep bore and is relatively free from microbiological contamination. What pathogenic microbial species are found in water supply to pharmaceutical firms? What challenges does it pose to the water purification process in these plants? What are the critical steps in purification process of water? These are some of the questions, answer to which is not available in literature. To find answers, this research work has been taken up.

The main objectives of this study are (a) To identify the extent and variation of pathogenic microorganisms in water source to pharmaceutical companies. (b) To identify the challenges it poses to purification process and (c) To develop a criticality hierarchy of purification process steps.

Methodology

The research was undertaken to assess the extent of microbial contamination in raw water and evaluate the water treatment process in the pharmaceutical industry. Methodology comprises of several steps. In the first step the sample from the water streams supplying water to Pharmaceutical companies located in east and south districts of Sikkim has been collected. In all a total of 60 spring water samples, from 20 sites, during monsoon, post-monsoon and pre-monsoon have been collected. . Thereafter a detailed analysis of presence of disease-causing bacteria in water samples has been undertaken using the standard membrane filtration technique as illustrated in Mackie and McCartney (Collee *et al.,* 1996). Each sample of 100 ml has been filtered through a 47-mm diameter and 0.45-μm pore size cellulose acetate membrane filter grid (Millipore). Thereafter the membrane, using a sterile flat-ended forceps is shifted to the surface of MacConkey agar plates and which are then incubated at 37 °C. The bacterial isolates are then identified using established procedures of morphological and the cultural characteristics. Further confirmation is also undertaken using biochemical characteristics of the isolates. The results of microbial analysis have been discussed with the quality assurance managers of fourteen pharmaceutical companies. It was found that conclusions in this respect are consistent with their experiences and records and that it does pose serious challenges to produce pharmaceutical formulations, especially water for injection. Hereafter, a brain-storming session has been held to identify critical process components in ensuring that final product is free from microbes and endotoxins. Later Risk Priority number (RPN) of each process component has been calculated by collecting data from fourteen quality experts. As this is an expert survey and no statistical analysis is being undertaken, the sample size of fourteen is sufficient (Clarke and Braun, 2013; Fugard and Potts, 2014; Guest *et al.*, 2006).

Results and Discussion

Details of pathogenic microorganisms found in water samples have been provided in Table 1.

From the table it can be seen that microbial pollution is water streams is fairly high and in all presence of 13 species of microorganisms is confirmed. Further, their presence remain high throughout the year despite having a spike during monsoon season. *Escherichia coli*is the most commonly found pathogenic bacteria and is found in as many as 43 samples. In terms of percentage it is present in 71.67% of the total samples of water collected. *klebsiella spp*, another common pathogenic bacteria has had a presence in almost 66.7% of the samples. *Citrobacter spp*, the third most common one has a relative lower presence and is reported from 18.34% of our samples. The least common ones include *Morganella morganii* and *Aeromonas hydrophilia* which are found in 3.33% each in collected samples. Comparatively presence of pathogenic bacteria is much higher in east Sikkim, than in South Sikkim and which can be explained through higher anthropogenic pressures in the east district. Density of population in east Sikkim is 257 and that in south Sikkim is 175. Microorganisms are a direct result of fecal contamination – again pointing towards anthropogenic demands.

Having established high microbial contamination of water, a one to one interaction was taken place with 14 quality executives working in pharmaceutical companies functioning in the state. All agreed that our findings are consistent with their own quality control records. As in most other parts of India these executives were used to ground water that is drilled out from bore-wells or on municipality supplied water, and which are not so high on microbial contamination, surface water at Sikkim poses a distinct challenge. The entire process of water purification is highly dependent on the quality of water at the source and this implies that the process at Sikkim needs to be observed and validated critically to ensure that final products are within the parameters set by WHO and other regulatory bodies.

In order to understand the possible errors in process steps that need detailed analysis in this respect have been identified through a brain storming session held with the fourteen quality assurance experts. These are:

a. Weather influence on the quality of the source water
b. Sodium Hypochlorite dosing missed completely

	East Sikkim						South Sikkim						
	Monsoon		Post-Monsn		Pre-Monsn		Monsoon		Post-Monsn		Pre-Monsn		
Microbe	**N.**	**%**	**N.**	**%**	**N.**	**%**	**N.**	**%**	**N.**	**%**	**N.**	**%**	**Tot**
Escherichia coli	9	90	8	80	7	70	7	70	7	70	5	50	**43**
klebsiella spp	8	80	8	80	7	70	6	60	5	50	6	60	**40**
Citrobacter spp	3	30	1	10	2	20	2	20	2	20	1	10	**11**
Shigella spp	2	20	3	30	1	10	1	10	0	0	1	10	**8**
Salmonella spp	2	20	3	30	2	20	1	10	0	0	0	0	**8**
Proteus spp	1	10	2	20	1	10	1	10	2	20	1	10	**8**
Pseudomonas spp	2	20	3	30	1	10	3	30	0	0	0	0	**9**
Acenitoacter	2	20	0	0	0	0	0	0	1	10	0	0	**3**
Enterobacter	1	10	1	10	2	20	2	20	1	10	1	10	**8**
Providencia	1	10	1	10	2	20	0	0	1	10	0	0	**5**
Morganella morganii	1	10	1	10	0	0	0	0	0	0	0	0	**2**
Serratia	2	20	0	0	1	10	0	0	1	10	1	10	**5**
Aeromonas hydrophil	0	0	0	0	1	10	1	10	0	0	0	0	**2**
Total	**34**	**21.3**	**31**	**19.38**	**27**	**16.9**	**24**	**15**	**20**	**12.5**	**16**	**10**	152

c. Sodium Hypochlorite concentration low
d. Pipe induced microbial growth
e. System is circulating or one-way (Dead leg)
f. Temperature of the system is suboptimal
g. Reverse Osmosis (RO) membrane malfunctioning
h. Employees do not comply with SOP
i. Untrained manpower
j. Delayed calibration of measurement tools
k. Sampling frequency
l. UV light not functioning appropriately

In the next step Risk Priority number (RPN) of these processes has been calculated based on a survey of experts. As mentioned to calculate RPN values of three qualitative variables are gathered. These are severity, occurrence likelihood and detection likelihood. RPN is a product of the values these three variables. Instead of mean, median of the fourteen values has been arrived at for final calculations and the details are provided in Table 2.

From the table we find that the most critical error is if sodium hypochlorite dosing is missed completely 9RPN 320). Severity on a scale 1 to 10 is rated as ten by the experts as this can close down a plants for months or even for years. This happened at a plant at Dubai a few years back and resulted in a massive loss. Occurrence likelihood is valued at 4, and which appears high. Detection likelihood is also weighed high at eight primarily because detection takes place only after microbial polluted water enters pipeline and where after it is practically impossible to undertake any corrective measures.

Second critical error is microbial growth in the pipe-line (RPN 192). This is high mainly because once microbial growth takes place inside pipeline, it is very arduous to remove them. Hence a value of 8 for severity is justified and so is the occurrence likelihood (valued 6). Water-borne microorganisms are frequently present and differ in their inclination to subsist and breed under different conditions. Inside pipelines bio-films are formed. A biofilm formation is attributed to bacterial cells that attach themselves to internal surface of pipeline and then exude glycocalyx (hydrated polymeric slimy matrices). The glycocalyx assists each bacterium to cover itself inside a protective sheath and survive. This biofilm matures with time and clump of cells are released. Bacterial cells can increase to 1000 in a mere seven days. Detection is also low because collected water may appear relatively free from microbial contamination even as biofilms are developing inside the pipeline. To counter this it is suggested to have break-tanks (to prevent purified water from reentering the water supply), using activated charcoal (to remove low-molecular-weight organic materials), using water softeners and deionization devices. Pipeline design plays a crucial role. Smooth pipelines made of appropriate material (electro-polished stainless steel) with minimum bends can help a lot. Avoiding water stagnancy and maintaining water temperature at 60^{o} to 70^{o} C, avoidance of leakages are some other measures.

Next critical error is RO membrane malfunctioning (RPN 150). Malfunctioning is attributed to loosely fitted system parts, depleted filters and worn-out membranes and wrong installation of a membrane. Severity is kept at 5, even though if purified water is to be used for water for injection. However occurrence likelihood and detection likelihood are high as occurrence takes place often and detection is not instantaneous.

In criticality subsequent error are Sodium Hy-

SN	Process component	Severity	Occurrence Likelihood	Detection Likelihood	RPN
1	Weather influence on the quality of the source water	7	3	1	21
2	Sodium Hypochlorite dosing missed	10	4	8	320
3	Sodium Hypochlorite concentration low	4	6	3	72
4	Pipe induced microbial growth	8	6	4	192
5	System is circulating or one-way (Dead leg)	2	1	2	4
6	Inadequate Temperature of the system	3	6	3	54
7	Reverse Osmosis (RO) membrane malfunctioning	5	6	5	150
8	Employees do not comply with SOP	3	2	6	36
9	Untrained manpower	6	1	2	12
10	Delayed Calibration of measurement tools	4	5	3	60
11	Inadequate Sampling frequency	2	4	1	9
12	UV light not functioning appropriately	2	4	5	40

pochlorite concentration being low (RPN 72), Delayed calibration of measuring systems (60), Inadequate temperature in the system (54), UV lights not functioning appropriately (40), Employees do not comply with SOP (36), Weather influence on the quality of the source water (21), Untrained manpower (12), Inadequate Sampling frequency (9), System is circulating or one-way (Dead leg) (04).

Conclusion

This study had been undertaken to identify the extent of pathogenic microbial pollution to which Pharmaceutical industries in Sikkim are subjected to, and the challenges it poses to their water purification process. Our findings in this respect are that presence of pathogenic microbes in feed water to pharmaceutical country is extremely high. While prioritizing the purification water process steps using risk priority number, determined on the basis of expert opinion of quality assurance managers. Missing of Sodium Hypochlorite dosing emerged as the most critical step in this respect, followed by Pipe induced microbial growth and malfunctioning Reverse Osmosis (RO) membrane malfunctioning. Implications for the quality professionals and regulatory authorities are that the extent of microbial pollution is extremely high and that some steps in purification process are highly critical and validation process should take this into consideration. It is to be ensured that

The water used in pharmaceutical industries should be frequently analyzed to preclude any possibilityof spreading of microorganisms.This study underlines the imperative need in the continuous monitoring of the water purification performance. To achieve this the washing of (storage tanks) reservoirs, deionization columns, reverse osmosis membranes, as well as the sanitation of distribution circuits should be undertakenon the basis of a schedule established for quality assurance.

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