biology and life sciences forum

MDPI

Proceedings

Assessing the Likelihood of *Staphylococcus aureus* Contamination in Bottled Drinking Water Production†

Patcharee Chittaphithakchai[1], Nonlapan Chomphulao[2], Pasika Kantasap [2], Kanyapak Wongput[2], Pawaret Kaitpoka[2], Thit Leesurapong[2], Thunnop Loakuldilok[2], Pichaya Poonlarp[3], Sutee Wangtuei[5], Wachira Jirarattanarangsri[4], Suthat Surawang[6] and Sukhuntha Osiriphun[4,*]

[1] Department of Medical, Regional Medical Medical Sciences Center5, Ministry of Public Health, Samut Songkhram, 75000 Thailand; Patcharee.c@dmsc.mail.go.th
[2] Division of Marine Product Technology, Agro-Industry, Chiang Mai University, Chiang Mai, 50100, Thailand; Thunnop.l@cmu.ac.th
[3] Division of Food Engineering, Agro-Industry, Chiang Mai University, Chiang Mai, 50100, Thailand; Pichaya.aey@hotmail.com
[4] Division of Food Science and Technology, Agro-Industry, Chiang Mai University, Chiang Mai, 50100, Thailand; Wachira.j@cmu.ac.th
[5] College of Maritime Studies and Management, Chiang Mai University, Samut Sakhon, 74000 Thailand
[6] Divisionof Product Development Technology, Agro-Industry, Chiang Mai University, Chiang Mai, 50100, Thailand; Suthat.s@cmu.ac.th
* Correspondence: Sukhuntha.o@cmu.ac.th; Tel.: +6653 94 82 16
† Presented at the title, place, and date.

Abstract: This study's objectives were to evaluate the possibility of *S. aureus* contamination in bottled drinking water and to determine the elements that affected the level of *S. aureus* in raw water. In two drinking water treatment facilities, samples of raw water, soft water, reverse osmosis (R.O.) water, and finished water were taken at various stages. In addition, raw water samples and risk indicators like pH, temperature, and residual chlorine were gathered at the packaging facility during the washing process. For Factory A (small scale), the pH values for the raw water, soft water, R.O. water, and finished water samples were 7.17, 7.24, 6.69, and 5.92, respectively. For Factory B (medium scale), the pH values were 7.9, 7.44, 6.97, and 6.8. All water samples from Factory A (2 CFU/ml) and Factory B (1 CFU/ml) had *S. aureus* concentrations that were within the acceptable range for human consumption. All water samples from Factory A (2-26 CFU/mL) and Factory B (11-316 CFU/mL) contained total coliforms as well. Our study revealed that *S. aureus* contamination in water was mostly caused by the pH and processing time. To prevent pathogen contamination in bottled drinking water, it is recommended that raw and finished water be kept at a pH level between 6.5 and 7.5.

Keywords: Risk Estimation; Food Risk Assessment; Sensitivity Analysis

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Published: date

1. Introduction

About 50–70% of a person's total weight is made up of water, which is essential for maintaining healthy cells, tissues, and organs (EPA, 2018) [1]. The source of drinking water may contain a variety of radionuclides, microorganisms, and chemicals. These dangers may result in gastrointestinal disorders, brain system or reproductive issues, as well as long-term conditions like cancer. Typhoid fever and cholera are examples of bacteria that cause waterborne disease. Diarrhea is one of the most typical negative effects of contaminated water and can be spread by drinking it [2]. One of the most frequent causes of food poisoning, *Staphylococcus aureus*, produces enterotoxin from an initial concentration of 100 organisms per milliliter of water. This pathogen has been found in a variety of places, including human nasal passages, skin, clothing, food, flies' digestive tracts, dust, and moisture droplets. As a proven source of their origin in drinking water supplies, *S. aureus* can be discovered in rural drinking water. Residents exposed to contaminated water may become colonized by *S. aureus* from drinking water. [3]. Individuals are exposed to avoidable health risks when water and sanitation systems are improperly managed. According to some studies, the prevalence of Escherichia coli and Staphylococcus aureus was 54.17% and 16.67%, respectively, with an average concentration of 1.04 log CFU/ml and 0.26 log CFU/ml in the drinking water filtration dispenser toll machines water in the Mahasarakham province of Thailand [4]. The characteristics of the contaminants in the water source determine the treatment procedure for disinfecting drinking water to decrease the prevalence of waterborne diseases [1]. Reverse osmosis (R.O.), charcoal filtration, and UV light are used to treat the water. According to the [5], R.O. can eliminate 99% of the germs in tap water as well as organic and inorganic pollutants. Drinking water may become quickly re-contaminated by other factors, particularly poor process hygiene brought on by erratic maintenance [6]. This can result in the contamination of the water with hazardous chemicals and dangerous microbes. In order to establish the high-risk parameters of processing steps as part of risk assessment, probabilistic modeling and sensitivity analysis of crucial control points in food processing and food safety systems are used [7]. Risk assessors can also utilize these models to help them make management decisions that will lower the risk of contracting foodborne illnesses [8]. In order to clarify the current state of the risk parameters for bottled drinking, it is required to apply modeling of the probability of *S. aureus* in terms of risk assessment of drinking water.

2. Materials and Methods

2.1. Water Sample

2.1.1. Sample collection

The physicochemical and microbiological tests were performed on water samples from one packing house (n = 5) and bottled drinking water (n = 3 at each step) from two factories (Factory A represents a small-scale factory and Factory B represents a medium-scale plant). The bottled drinking water processing facility took samples of raw water, soft water, reverse osmosis water, and finished water. The samples were maintained in iceboxes after sample collection and transported to the lab (Department of Medical, Regional Medical Medical Sciences Center5, Samut Songkhram) within two hours. In triplicate, each sample was taken and examined.

2.2. Physicochemical analysis

The AOAC official technique was used for the physicochemical evaluations of all water samples (AOAC, 2005) [9]. The packing house gathered and evaluated the water sample's pH, residual chlorine (ppm), temperature (^{o}C), and processing time. Using a pH meter (Hanna Instruments, USA), water samples from bottled drinking water were analyzed to determine the pH at each stage.

2.3. Microbiological analysis

All samples were collected for measurement of the quantification and risk of *S. aureus* and total coliforms by following the FDA BAM, 2019 (Chapter 12) 10] and FDA BAM (Appendix 2) [11] procedures.

2.3.1. Plating media method of S. aureus and Total Coliforms

A quantity of 25 ml of samples was measured, rinsed in a 250 mL bottle containing 225 mL of sterile 0.1% peptone water, and then the suspension diluted from 1:10−2 to 1:10−4. A volume of 0.1 mL of each dilution was spread on Braid Parker agar plates (BPA) (Merck, Germany) for the enumeration of *S. aureus* and Five tubes should be used for each dilution of the multiple-dilution MPN series used to enumerate of total coliforms using Violet Red Bile Agar (VRBA) (Merck, Germany). The plates were incubated at 37°C for 24 h for isolation of bacteria (FDA BAM 2019 [10] and FDA BAM 2020 [12]). The colony-forming units of characteristic *S. aureus* and total coliforms were counted and reported as CFU.

2.4. Sensitivity analysis

To explain how *S. aureus* became contaminated during the various processing steps, a probabilistic risk assessment (quantitative model) was developed. Using the analysis tool program in Microsoft ExcelTM, the results of the microbiological analysis were transformed into log10 units and put through a straightforward regression analysis. It often entails fitting a relationship between inputs and outputs, such as the following linear one [13].The equation is as follows:

$$Yi = bo + b1X1,i + b2X2,i + \ldots + bmXm,i + ei \quad (1)$$

Yi is the ith output data point for the ith input data point, Xj,i is the ith input data point for the jth input, bj is the jth input's regression coefficient, and ei is the ith data point's error. The basis function for each term in the regression model may be distinct and either linear or nonlinear. According to Frey et al. (2003) [13], the regression coefficient, bj, for a linear model can be understood as the change in the output Yi when the input Xj,i for a given value of j increases by one unit and the values of all other inputs remain constant. Rank order correlations, a non-parametric statistic for estimating the correlational link between two means, were employed in the regression sensitivity analysis based on Spearman's rank correlation calculations The analysis involved running simulations with probability distributions allocated to the inputs and determining the impact of input variance on the output distribution. Then, tornado graphs might be produced. The various input factors were represented by horizontal bars on the graph, and the length of the bars indicated the degree of association with the mean numbers and the amount of *S. aureus* discovered in the samples (output variables). The nominal rage sensitivity analysis equation was as follows [13]:

$$\text{Sensitivity} = \frac{\text{Output at maximum input value - Output at minimum input value}}{\text{Output at nominal input value}} \quad (2)$$

2.5. Statistical analysis

All measurements were carried out in triplicate. The means and standard deviation (± SD) were calculated using Data Analysis in the Excel platform.

3. Results

3.1. Physicochemical and microbiological properties of the water samples

Table 1 displays the conditional factors for the bottling plant (Plant B) and the packing facility (Plant A). The packing house had water with pH values between 10.33 and 10.81, residual chlorine concentrations between 0.23 and 1.67 ppm, and temperatures between 17.3 and 19.1°C. In order to generate the simulation models, the quantity of S. aureus (3.40 LogCFU) discovered in the water samples was evaluated. Raw, soft, R.O., and finished water had pH values of 7.17, 7.24, 6.69, and 5.92, respectively, for plant A. For plant B, the corresponding values were 7.60, 7.44, 6.97, and 6.80. S. aureus concentrations at Plant A and Plant B ranged from 1-3 CFU. Both Factory A (2-26 CFU/mL) and Factory B (11-316 CFU/mL) provided water samples that also included total coliforms.

Table 1. Physicochemical and microbiological analysis results of water sample.

Plants	Packing House	Drinking Water Factory A				Drinking Water Factory B			
Samples	Water	Raw	Soft	R.O.	Finished	Raw	Soft	R.O.	Finished
Time (min)	0-20	-	-	-	-	-	-	-	-

Temperature (°C)	17.3−19.1	-	-	-	-	-	-	-	-
pH	10.33−10.81	7.17±0.25	7.24±0.26	6.69±0.21	5.92±0.32	7.60±0.33	7.44±0.29	6.97±0.22	6.80±0.21
Residual chlorine (ppm)	0.23−1.67	-	-	-	-	-	-	-	-
Total coliform (CFU)	-	189±8.60	316±44.22	30±44.40	145±7.72	26±3.89	2±3.13	20±1.48	13±1.47
***S. aureus* (CFU)**	3.40 LogCFU	3±1.00	1±0.00	1±0.00	1±0.00	1±0.00	1±0.00	1±0.00	1±0.00

Note: The numbers are means±S.D. from three independent replicates.

3.2. Probabilistic distribution

The pH of the water and the concentration of S. aureus and total coliforms in the water of Plants A and B were simulated to have probability distribution values of Normal(1.5,1.0) and Normal(1,0) and Normal(163.09,89.10) and Normal(13.97,6.70), respectively. Water from Plants A and B had normal pH distributions of 6.75, 0.61 and 7.20, 0.38, respectively. The concentration of S. aureus and total coliforms in water samples of factory A and B was in the range of 0-4 CFU and 0-2 CFU and 0-316 CFU and 0-30 CFU, respectively, with the highest probabilities of S. aureus and total coliforms contamination in the water of Plant A and Plant B being 0.42 and 0.35 and 4.47E-03 and 0.0514, respectively. The pH values in the water of Plants A and B had the maximum probabilities of 0.6 at pH ranging from 5-8 and 1 at pH ranging from 6-8, respectively.

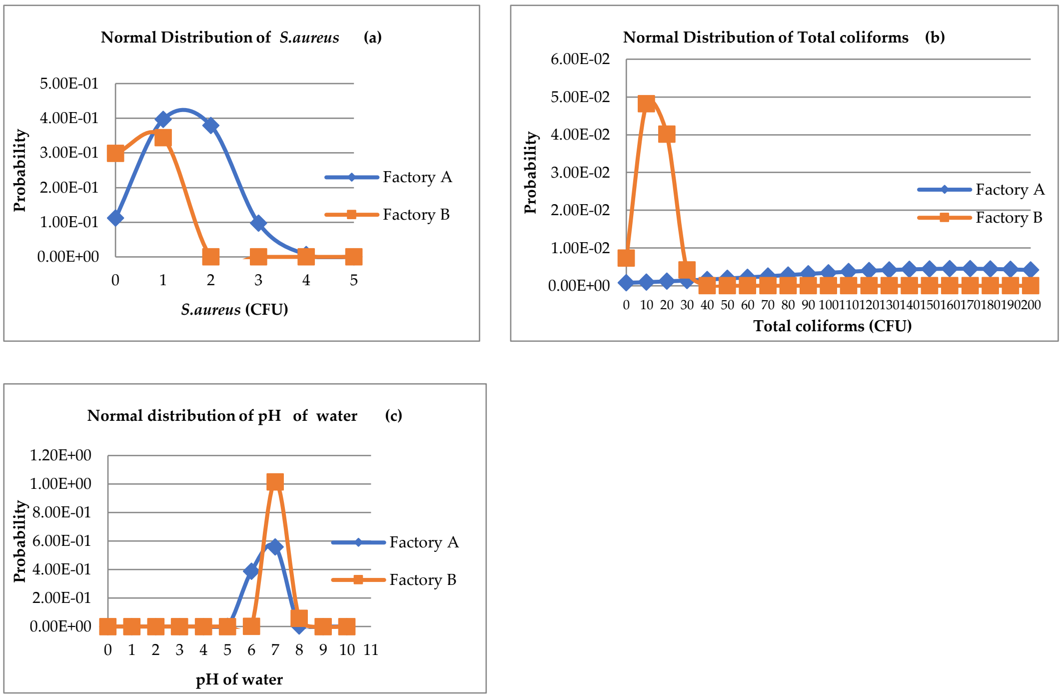

Figure 1. (a) The probability distribution functions of S. aureus concentrations in water (b) The probability distribution functions of total coliforms concentrations in water samples. (c) The probability distribution functions of pH values of water samples.

3.3. Modeling with multiple regression equations

The sensitivity analysis of water was developed using the equation for multiple regression, and the equations are as follows for the multiple regression of S. aureus contamination in water (eq. 3) and S. aureus contamination in bottled drinking water (eq. 4):

$$Y = -233.9910+(1.8471\times \text{Time})-(9.1343\times \text{Temperature of Water})-(5.0746\times \text{Residual Chlorine in Water})+(40.5970\times \text{pH of Water}) \quad (3)$$

$$Y = -0.1260+(0.1972\times \text{pH of Water}) \quad (4)$$

The model's input parameters included the water's temperature, pH, residual chlorine content, and temperature. The model's result was the detection of S. aureus water contamination.

3.4. Sensitivity Analysis

The Excel Platform was used to do the sensitivity analysis. For each input variable, horizontal bars on a tornado chart were drawn, with the length of the bars indicating the strength of the association with the output variable.

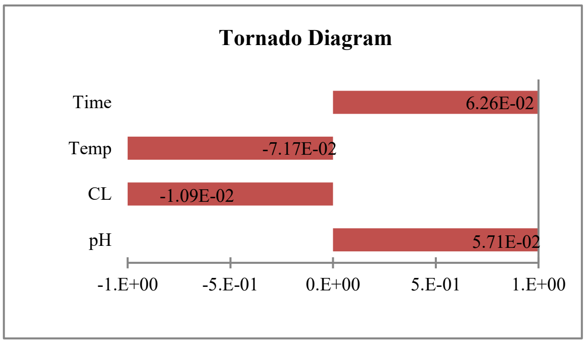

Figure 2. Sensitivity analysis of the risk factors of S. aureus contaminated in water.

The sensitivity analysis results (Figure 2) show that the temperature of the water and residual chlorine concentration were the conditional parameters affecting a decrease in the number of *S. aureus* in the water and an increase in the concentration of *S. aureus* in the water. Water temperature and residual chlorine concentration were the main intervention measures that helped control the effect on the water.

4. Discussion

To assess the health risk of drinking water, the study of Wibuloutai and coleagues, which assesses the microbiological quality of drinking water produced by drinking water filtration dispenser toll machines (DFTMs) was compared to this study. 210 samples in total were chosen at random from 70 DFTMs that were spaced 500 meters apart from Mahasarakham University. The DFTM water had high levels of Escherichia coli and Staphylococcus aureus, with prevalence rates of 54.17% and 16.67% and average values of 1.04 log cfu/ml and 0.26 log cfu/ml, respectively. The risk assessment used the @Risk tool to calculate the likelihood of exposure as 1.67 E-01 and the odds of getting sick from S. aureus and E. coli as 2.08 E-03 and 1.58, respectively (@Risk is a software tool to generate a distribution of possible outcomes and estimate the probability of different levels of pathogen exposure). [4] It has been established that the findings of our investigation may be safely consumed. The suggested interventions keep the water's pH between 6.5 to 7.5 since doing so could help to lessen the amount of microbial contamination in the water [15]. According to Pratum and Khananthai (2017) [14}, the quality of drinking water also depends on the tap water or water source used throughout the manufacturing process. The local government must issue local laws for the control of such businesses in the area of responsibility in accordance with the Ministry of Public Health's notification on hazardous health activities [4].

5. Conclusions

This study was successful in identifying the potential risk of S. aureus contamination in water as well as the recommended pH levels for processing bottled drinking water at small- and medium-sized facilities. The findings of the sensitivity study revealed that the pH of the water samples played a significant role in the procedure. This result implies that preserving the pH of the water throughout the procedure, as shown by this study, is a helpful technique for lowering the risk of contamination. However, harmful bacteria have the potential to taint finished goods if the water quality is inadequate.

Author Contributions: "Conceptualization, P.C.,N.C.,P.K.,K.W.,P.K.,T.L.,T.L.,P.P., S.W., W.J., S.S.,and S.O..; methodology, P.C., N.C.,P.K.,K.W.,P.K.,T.L.,T.L.,P.P., S.W., W.J., S.S.,and S.O.; software, P.C., N.C.,P.K.,K.W.,P.K.,T.L.,T.L.,P.P., S.W., W.J., S.S.,and S.O.; validation, P.C., N.C.,P.K.,K.W.,P.K.,T.L.,T.L.,P.P., S.W., W.J., S.S.,and S.O.; formal analysis, P.C., N.C.,P.K.,K.W.,P.K.,T.L.,T.L.,P.P., S.W., W.J., S.S.,and S.O.; investigation, P.C., N.C.,P.K.,K.W.,P.K.,T.L.,T.L.,P.P., S.W., W.J., S.S.,and S.O.; resources, P.C., N.C.,P.K.,K.W.,P.K.,T.L.,T.L.,P.P., S.W., W.J., S.S.,and S.O.; data curation, P.C., N.C.,P.K.,K.W.,P.K.,T.L.,T.L.,P.P., S.W., W.J., S.S.,and S.O.; writing original draft preparation, P.C., N.C.,P.K.,K.W.,P.K.,T.L.,T.L.,P.P., S.W., W.J., S.S.,and S.O.; writing—review and editing, P.C., N.C.,P.K.,K.W.,P.K.,T.L.,T.L.,P.P., S.W., W.J., S.S.,and S.O.; visualization, P.C., N.C.,P.K.,K.W.,P.K.,T.L.,T.L.,P.P., S.W., W.J., S.S.,and S.O.; supervision, P.C., N.C.,P.K.,K.W.,P.K.,T.L.,T.L.,P.P., S.W., W.J., S.S.,and S.O.; project administration, P.C., N.C.,P.K.,K.W.,P.K.,T.L.,T.L.,P.P., S.W., W.J., S.S.,and S.O.; funding acquisition, P.C., N.C.,P.K.,K.W.,P.K.,T.L.,T.L.,P.P., S.W., W.J., S.S.,and S.O.; All authors have read and agreed to the published version of the manuscript."

Funding: "This research received no external funding" or "This research was funded by the Department of Medical Sciences, Regional Medical Sciences Center 5, 7, 11, Ministry of Public Health, Thailand, grant number 006 FM 0117"

Acknowledgments: The Department of Medical Sciences at Samut Songkhram (Thailand) is acknowledged. Thank you to Regional Medical Sciences Center, Ministry of Public Health, Thailand for the insightful advice and useful details you supplied for this study. The Department of Medical Sciences, Samut Songkhram Regional Medical Sciences Center, Ministry of Public Health, Thailand, financed this study. & Chiang Mai University's Faculty of Agro-Industry in Chiang Mai, Thailand. Chiang Mai University provided some funding for this study.

Conflicts of Interest: "The authors declare no conflict of interest."

References

1. EPA, 2018, Drinking Water Quality. Available online: https://www.epa.gov/report-environment/drinking-water (accessed on 8 September 2023).
2. World Health Organization (WHO), 2020, Water and health, Available online: https://www.who.int/news-room/fact-sheets/detail/drinking-water (accessed on 8 September 2023)
3. Lechevallier, M.W., and Seidler, R.J. Staphylococcus aureus in Rural Drinking Water, Department of Microbiology, Oregon State University, Corvallis, Oregon 97331, 1980,
4. Wibuloutai, J.;Thanomsangad, P.; Benjawanit, K.; Mahaweerawat, U.; Microbial risk assessment of drinking water filtration dispenser toll machines (DFTMs) in Mahasarakham province of Thailand, *Water Supply*. **2019**, 19, 1438–1445. doi.org/10.2166/ws.2019.016
5. World Health Organization (WHO) 2011 Guidelines for Drinking Water Quality, 4th edn. World Health Organization, Geneva, Switzerland. Available online: https://www.who.int/publications/i/item/9789241549950. (accessed on 8 September 2023).
6. Ali S. S.; Anwar Z., Zaman J., Khattak K. and Islamic I. Microbial analysis of drinking water and water distribution system in new urban Peshawar. *Current Research J. of Bio. Sci.* **2012**, 4, 731–737.
7. Codex Alimentarius Commission (2014). Principles and Guidelines for the Conduct of Microbiological Risk Assessment. Available online: http://www.fao.org/docs/eims/upload/215254/CAC_GL30.pdf. (accessed on 8 September 2023)
8. Mishra, N.; Mishra, A. K., & Bidija, M. A study on correlation between depression, fear of fall and quality of life in elderly individuals. *Int. J. of Research in Medical Sci,* **2017**, 5(4), 1456–1460. https://doi.org/10.18203/2320-6012.ijrms20171245.
9. AOAC, Official methods of analysis of the association of analytical chemists international, eighteenth ed. Association of Official Analytical Chemists, Arlington. 2005.
10. Tallent,S.; Hait, J., Reginald W. B., and Gayle A. L., Blodgett R., 2010. BAM appendix 2: Most probable number from serial dilutions. Available online: https://www.fda.gov/food/laboratory-methods-food/bam-appendix-2-most-probable-number-serial-dilutions#tab5. (accessed on 8 September 2023).
11. Feng, P., Stephen D. W., Michael A. G., Burkhardt,W., 2020, BAM Chapter 4: Enumeration of *Escherichia coli* and the Coliform Bacteria, https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4-enumeration-escherichia-coli-and-coliform-bacteria. (accessed on 8 September 2023)
12. Frey, C.H., Mokhtari, A., Danish, T. Evaluation of Selected Sensitivity Analysis Methods Based Upon Applications to Two Food Safety Process Risk Models.US.Department of Agriculture. 2003; 460 pages.

13. Plant Standard and Certification Division, Thailand Agricultural Regulations and Standards for Plants Certification, Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand. 2013, 345 pages.
14. Pratum, C; and Khananthai, N., Assessment of Factors Affecting Drinking water quality from Free Water, *Int. J. of Environ and Sci Ed.* **2017**, 12(4), 787-797.