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Research Article

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2024.55.3.499-503

Evaluation of physico-chemical quality and occurrence of Salmonella in water from food retail outlets#

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Citation: Anjali, S., Menon, K.V., Sunil, B., Vijay, D. and Sankar, S.2024. Evaluation of physico-chemical quality and occurrence of Salmonella in water from food retail outlets. *J. Vet. Anim. Sci.* **55** (3):499-503

Received: 13.10.2023 Accepted: 27.01.2024 Published: 30.09.2024

Abstract

Salmonella is one among the most important biological contaminants in drinking water that possess safety hazards to human life. Faecal contamination of the water, indirectly from the household sewage discharge, municipal sewage etc. attributes to the repeated detection of Salmonella in water. This study aimed to investigate the occurrence of Salmonella and physico- chemical quality evaluation of samples of water collected from food retail outlets of Thrissur and Kollam districts. A total of 50 water samples (25 each from Thrissur and Kollam districts) used for cooking purpose were collected over a period of six months, March to August 2023. Water samples were subjected to comparative evaluation of physico-chemical qualities viz., pH, turbidity, total dissolved solids (TDS) and hardness. Collected water samples were examined for Salmonella spp. by conventional culture method and PCR. Genus specific and virulence genes of the isolates were detected using polymerase chain reaction. The occurrence of Salmonella spp. *in samples of water collected from Thrissur was 24 per cent whereas in Kollam district it was only four per cent. The overall occurrence of Salmonella spp. in samples of water collected from both districts was 14 per cent. Physico-chemical parameters of water samples viz., pH, turbidity, total dissolved solids and hardness were within the BIS prescribed standards. The molecular characterisation of Salmonella spp. detected 16S rRNA in all isolates but invA and spvA genes were detected only in 14.28 per cent of the isolates. Hence proper disinfection of water and food sanitation in the retail outlets will help to minimize the risk of foodborne pathogens.*

Keywords: *Salmonella, water, physico-chemical quality, food retail outlets*

Water is one of the most valuable resources in our planet which is very essential for the existence of life. About two billion people live in water-stressed regions, which is expected to be exacerbated as a result of population growth and climate change. Globally, at least 1.7 billion people use a drinking water source which is contaminated with faeces (WHO, 2023). The microbial contamination of drinking water poses the greatest risk to drinking water safety, which can transmit diseases and is estimated to cause approximately 5,05,000 diarrhoeal deaths each year (WHO, 2023). The occurrence and abundance of these microorganisms in_water are strongly influenced by physico-chemical factors. Salmonella are Gram-negative facultative anaerobic bacteria which are commonly isolated from humans, animals, and the environment

#Part of MVSc thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

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(Wegener, 2012). They continue to be a serious global public health issue and are the primary cause of acute gastroenteritis across the globe in a number of nations. Hence the study was undertaken to study the occurrence of Salmonella in water from food retail outlets from Thrissur and Kollam districts of Kerala and to evaluate the physico-chemical quality of the water samples.

Materials and methods

A total of 50 water samples, 25 each from retail outlets of Thrissur and Kollam districts were collected in sterile sample containers (approximately 250mL) which was transported to the laboratory in thermocol containers and immediately processed. The physico-chemical parameter like pH was determined using Cyberscan pH/ mv/c meter, turbidity using Spectroquant colorimeter move 100, total hardness using water testing kit and total dissolved solids (TDS) using Digital multiparameter water analyser. All the water samples collected were subjected to isolation and identification of *Salmonella* spp. (APHA, 2012). About 50 mL of water sample was filtered through 0.45 µm pore size membrane filter and the membrane were immersed in 50 mL tetrathionate broth and incubated at 35°C for 24 h. After incubation a loopful was plated on MacConkey agar and incubated at 37°C for 18 h. Non-lactose fermenting transparent colonies was selected and plated on xylose lysine deoxycholate (XLD) agar. The isolates were identified by biochemical tests (Barrow and Feltham, 1993). All the biochemically confirmed isolates were subjected to extraction of DNA using boiling and snap chill method (Ram *et al*., 2019) and PCR for detection of genus specific 16S *rRNA* (Kaabi and AL-Yassari, 2019) and virulence genes *viz*., i*nv*A (Bhatta *et al.,* 2007) and *spv*A (Amini and Mobasseri, 2019). The genomic DNA of *Salmonella* Enteritidis (ATCC-13076™) was used as positive control. Statistical analysis of the data was performed using SPSS 24.0 software.

Results and discussion

Physico-chemical quality of the water

Physico-chemical quality evaluation of the water samples which were used for cooking purpose from Thrissur and Kollam districts were analysed in the current study and the results are shown in Table 1. Statistical analysis using independent variable t-test was performed to determine the significance difference in physical quality parameters of water between two districts.

The mean pH of water samples from Thrissur and Kollam districts was 6.767 ±0.012 and 7.094 ±0.196 respectively. No significant difference was found between the pH of water samples collected from both districts. A similar finding of pH 6.83 to 7.34 was reported by Dinesh and Ganorkar (2013) from India. As per BIS standard (2012), in drinking water the permissible range of pH should be between 6.5 to 8.5. The pH of water obtained from both districts was within the prescribed limit. The pH is an important indicator of water quality as it shows acidity or alkalinity. It also indicates whether the water is safe for drinking or not.

The mean turbidity observed from Thrissur and Kollam district was 4.84 ±0.564 NTU and 3.84 ±0.512 NTU respectively. Aneesha (2019) from Kerala, found that the mean turbidity of well water samples in Thrissur district was 3.28±1.33 NTU. However, a higher turbidity value 0.078 to 121.9 NTU was reported by Vishnu *et al.* (2014) from Kerala. The acceptable turbidity range in drinking water as per BIS (2012) is 1 to 5 NTU. The mean turbidity observed from both districts was within the prescribed standard. Turbidity in water is due to the presence of materials like clay, silt, algae, organic and inorganic matter, dissolved organic compounds having colour and microscopic organisms. The determination of water turbidity is important as it provides an estimation of water contamination. The particles of turbidity protect microbes by reducing their exposure to attack of disinfectants.

The samples of water analysed from Thrissur and Kollam districts showed a mean hardness value of 109.80 ±2.327 mg/L and 126.80 ±1.934 mg/L. The hardness of water samples analysed was under the category of moderately hard to hard water. Dinesh and Ganorkar (2013) estimated a total hardness 102.8 to 194.4 mg/L from the water samples evaluated from India. As per BIS standard (2012), in drinking water the permissible level of hardness should be from 200 to 600 mg/L. Samples of water taken from both districts had hardness levels within the prescribed BIS standard. Hardness is an important parameter of water which decreases the toxic effect of poisonous elements, which is the measure of total calcium and magnesium ion concentration in a water sample.

Table 1. Mean physico-chemical quality of water samples collected from Thrissur and Kollam districts

Sl. No.	Parameters	Mean ± SE		t-value	p-value
		Thrissur	Kollam		
1	pH	6.767[a] ±0.012	7.094[a] ±0.196	1.168	0.249
2	Turbidity (NTU)	4.84[a] ±0.564	3.84[a] ±0.512	1.123	0.267
3	Hardness (mg/L)	109.80[a] ±2.327	126.80[a] ±1.934	1.312	0.196
4	TDS (mg/L)	101.94[a] ±1.821	114.71[a] ±1.863	0.981	0.332

Means bearing same superscript do not differ significantly within a row ($p<0.05$)

The water samples from Thrissur district had a mean TDS value of 101.94 ±1.821 mg/L whereas samples from Kollam district had a TDS value of 114.71 ±1.863 mg/L. A TDS value of 235.99±19.43 mg/L was reported by Aneesha (2019) from Thrissur, Kerala. However, a higher TDS value of 390-1007 mg/L was reported in India by Vaithyanathan and Ravichandran (2015). As per BIS (2012) standard, in drinking water the TDS should be between 500 mg/L to 2000 mg/l. The TDS value of the water from both districts were within the acceptable limit. The TDS of water is the total concentration of dissolved substances (organic and inorganic) in the water. High TDS levels are generally not hazardous, however those with heart and kidney conditions may be affected by high concentrations of these.

Occurrence of Salmonella spp. in water samples collected from Thrissur and Kollam districts

A total of 50 water samples was processed for isolation and identification of *Salmonella* spp. by enrichment in tetrathionate broth followed by selective plating on XLD agar. Typical pink colony with black centre were selected for biochemical characterisation which showed that Salmonella was present in 24 per cent and four per cent of water samples collected from Thrissur district and Kollam district respectively. The overall occurrence of *Salmonella* spp. in water samples collected from both districts was 14 per cent. A similar occurrence of 12 per cent and 14 per cent was reported by a study conducted by Rather *et al.* (2013) from India and Bhatta *et al.* (2007) from Nepal. However, Chandran *et al.* (2011) from India reported a higher occurrence of 87.5 per cent in water samples analysed. There was significant difference ($p<0.05$) between the occurrence of *Salmonella* spp. in water samples collected from Thrissur and Kollam district when statistically analysed using chi square test. (Table 2).

Molecular characterisation of Salmonella spp.

All the Salmonella isolates analysed were found to be positive for 16S *rRNA* gene. Among the seven isolates of *Salmonella* spp. detected from water, gene *inv*A was detected only in one isolate (14.28 per cent). Yanestria *et al.* (2019) from Indonesia reported a similar low prevalence of 12.5 per cent of *inv*A gene in *Salmonella* spp. However, a higher prevalence of 100 per cent was detected by Jisna (2022) from India. Some Salmonella are non-invasive, or they may have other invasive mechanisms, which could account for the low incidence of *inv*A in this study (Kadry *et al*., 2019). Gene *spv*A was detected in 14.28 per cent of the isolate. The PCR amplicons of all the three genes are shown in Fig. 1, 2 and 3. In *Salmonella* spp. *spv* locus is closely linked with the strains that cause non-typhoid

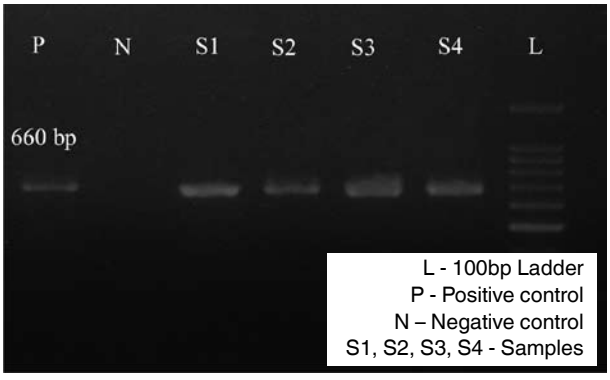

Fig. 1. PCR image of 16S *rRNA* gene

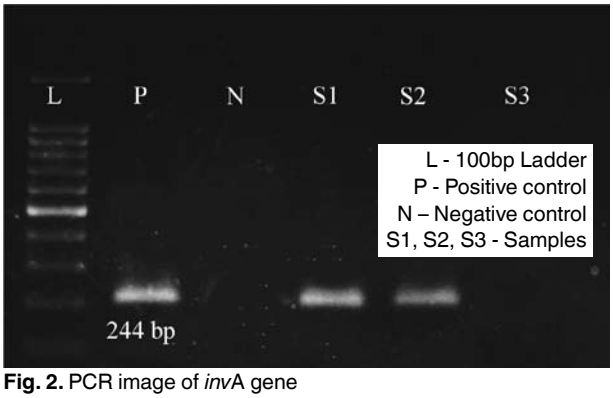

Fig. 2. PCR image of *inv*A gene

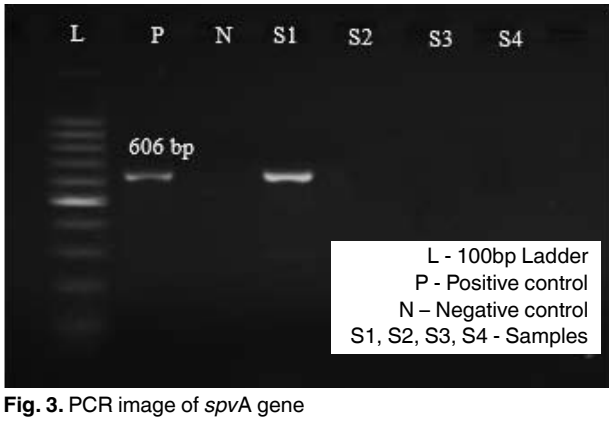

Fig. 3. PCR image of *spv*A gene

Table 2. Occurrence of *Salmonella* spp. in water samples collected from Thrissur and Kollam districts

Sl. No.	District	Sample analysed	Positive		Chi-square	p-value
			Number	Per cent		
1.	Thrissur	25	6[a]	24	4.153	0.042
2.	Kollam	25	1[b]	4		
Total		50	7	14		

Figures bearing different superscript (a - b) within a column differ significantly ($p<0.05$)

Table 3. Molecular characterisation of *Salmonella* spp.

Sl. No.	Genes	Isolates analysed	Positive	
			Number	Per cent
1.	16S *rRNA*	7	7	100
2.	*inv*A	7	1	14.28
3.	*spv*A	7	1	14.28

salmonellosis, but are absent in typhoid strains. The results are shown in Table 3.

Conclusion

This study revealed that Salmonella was present in water which indicates faecal contamination. Presence of the organism is a possible source of foodborne illness. However, the physico-chemical quality of water was within the prescribed limits. Hence there is need to create awareness among the personals involved in processing of foods in retail outlets on the hygienic and sanitary practices to be followed to reduce foodborne outbreaks.

Acknowledgement

This work was financially supported by Kerala Veterinary and Animal Sciences University, Kerala. The facilities are provided by Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Thrissur.

Conflict of interest

The authors declare that they have no conflict of interest.

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