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Article

Survey of microbial contamination levels of direct drinking water from terminal devices in Yantai City

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Abstract: The study aimed to assess the quality and sanitation of direct drinking water from terminal devices collected from various places such as communities, schools, and homes in Yantai City. This research was to support regulations, enhance authorized supervision, and inform consumer choices. 232 samples were randomly gathered in aforementioned places between June to November, 2019. The test of aerobic plate count, *Coliforms*, and *Pseudomonas aeruginosa* followed the standard operating procedure provided by Chinese National Food Contamination and Harmful Factors Risk Monitoring Manual in 2019. Findings showed that 84.05% of the samples had aerobic plate counts as main contamination, with *Coliforms* and *Pseudomonas aeruginosa* was respectively 3.02% and 7.33%. These results revealed that aerobic plate count was the main contaminant in high-quality drinking water, while *Pseudomonas aeruginosa* was the main pathogenic bacteria. Overall, 9.48% of the samples exceeded the standard. Family settings had the highest non-compliance rate at 12.68%, followed by schools at 8.97%, and communities at 7.23% by comparing different sources, yet these differences were not statistically significant ($\chi^2 = 1.36$, $P > 0.05$). There was no clear seasonal variation regularity of the detection rate. However, there was clear variation in monthly non-compliance rates. The highest was at 15.00% in November, followed by June at 13.89%, September at 11.76%, October at 6.25%, August at 5.88%, and July at 5.00%. Yet these differences were not statistically significant, either ($\chi^2 = 4.47$, $P > 0.05$). It was notable that some samples exhibited multiple contamination by various indicators. In summary, the study showed widespread contamination of direct drinking water by aerobic plate count, *Coliforms* and *Pseudomonas aeruginosa*, with aerobic plate count being the most prevalent issue.

Keywords: fine drinking water; microbes; contamination; investigation; analysis

Water is an indispensable nutrient for various life forms. When the body's water intake is insufficient or the water carries germs or toxic substances, it will lead to disease and even death [1,3]. Water quality safety and health are highly valued by the state and society, and people's requirements for drinking water quality are also constantly improving. Therefore, direct drinking purified water that has been deeply treated and purified by drinking water equipment is favored. Convenient, hygienic and safe direct drinking water is accepted by more and more communities, schools and families [4,5]. In particular, tap water is used as raw water. Terminal direct drinking water that has been deeply treated and purified by terminal direct drinking water equipment has become the first choice for families, schools and communities. However, at present, there is no national food safety standard for direct drinking water in China, and there is no unified supervision mode for the direct drinking water industry, which leads to the fact that the quality of direct drinking water cannot

be fully guaranteed. In order to understand the microbial sanitary status of terminal direct drinking water, the total number of *colonies*, *coliform* and *Pseudomonas aeruginosa* were monitored in the terminal direct drinking water of families, communities and schools, in order to master its pollution status and level, and provide basis for the formulation of direct drinking water standards, department supervision and residents' drinking safety.

1. Materials and methods

1.1. Sample source

According to the division of administrative regions, 12 monitoring points are set up, and each monitoring point has 3 sampling links (families, communities and schools). From June to November 2019, the direct drinking water prepared by the terminal direct drinking water equipment is taken as the collection object. A total of 232 samples are collected every month, covering 73 schools (39 primary schools, 32 middle schools, 2 universities), 54 communities and 50 families.

1.2. Sample collection method

According to the requirements of GB/T 5750.2-2006 [6] for the collection of terminal water, random sampling shall be conducted. Before sampling, apply a 75% alcohol cotton ball to the faucet outlet for disinfection. After the faucet is turned on for 5min under normal water discharge state, aseptically collect 0.5 L water sample and put it into a water sample collection bag (containing 0.4 mg sodium thiosulfate), store and transport it at low temperature, submit it for inspection, and test it within 4 h.

1.3. Test method

According to the inspection standard operating procedures [7] specified in the 2019 national food pollution and hazardous factor risk monitoring manual, the total number of *colonies*, *coliform* and *Pseudomonas aeruginosa* (Quantitative) were detected. Total bacterial count shall be in accordance with GB 4789.2-2016.

In 2016, the first method was plate counting method, and the detection limit of the method was 1 CFU/mL [8]; *coliform* group was detected by MPN counting method in accordance with GB 4789.3-2016 method 1, with detection limit of 3 MPN/100 mL [9]; *Pseudomonas aeruginosa* was detected by the filter membrane method in accordance with GB 85382016, and the detection limit of the method was 0 CFU/250 mL [10]. Below the method detection limit is defined as "not detected (ND)".

1.4. Judgment criteria

According to CJ 94-2005, *coliform* bacteria shall not be detected per 100 mL water sample as the judgment basis [11]. *Pseudomonas aeruginosa* is judged according to the limit value of 0CFU/250ml specified in GB 19298-2014 [12]. If one index of each water sample exceeds the specified limit value, it is determined as exceeding the standard sample.

1.5. Statistical analysis

Excel 2019 software is used for data entry, origin 2017 software is used for data statistical analysis, chi square test is used for rate test, and the test level is $a = 0.05$.

2. Results and analysis

2.1. Microbial pollution of direct drinking water

In 232 samples of terminal direct drinking water, the detection rates of total bacterial count, *coliform* group and *Pseudomonas aeruginosa* were 84.05%, 3.02% and 7.33% respectively. The total bacterial count pollution was the main microbial pollution factor, and the difference between the control groups was statistically significant ($\chi^2 = 447.19$, $P < 0.01$). The numerical range of total bacterial count is 0~140 CFU/mL, with a median of 58 CFU/mL; the median of *Pseudomonas aeruginosa* is 0 CFU/250 mL, with a numerical range of 0~140 CFU/250 mL (**Tables 1** and **2**); the maximum value of *coliform* group is 66 MPN/100 mL, with a median of 3 MPN/100 mL (**Tables 1** and **2**).

Comparison of sampling links: the total number of *colonies* and the detection rate of *Pseudomonas aeruginosa* from large to small: family > Community > school, comparison between groups, total number of *colonies* ($\chi^2 = 0.44$, $p > 0.05$), *Pseudomonas aeruginosa* ($\chi^2 = 0.24$, $p > 0.05$). *Coliform* bacteria were mainly detected in family and community samples, with detection rates of 7.04% and 2.56% respectively. The value range was 0~66 CFU/mL, and the median was 0 CFU/mL; no *coliform* bacteria were detected in school samples, comparison between groups, *coliform* bacteria ($\chi^2 = 6.30$, $p < 0.05$) the difference was statistically significant (**Table 1**).

Table 1. Detection rate of microorganisms in direct drinking water from terminals at different links.

Link	*N*	Total bacterial count			*Coliform* group			*Pseudomonas aeruginosa*		
		Detection rate	Median/ (CFU/mL)	Max/ (CFU/mL)	Detection rate	Median/ (MPN/mL)	Maximum/ (MPN/mL)	Detection rate	Median/ (CFU/250 mL))	Maximum/ (CFU/250 mL))
Family	71	85.92%	60	12,000	7.04%	ND*	66	8.45%	ND	140
Community	83	84.34%	50	11,000	0.00%	ND	13	7.23%	ND	40
School	78	82.05%	59	14,000	2.56%	ND	0	6.41%	ND	50
Total	232	84.05%	58	14,000	3.02%	ND	66	7.33%	ND	140

According to the analysis of the monitoring sample collection month, the detection rate of total bacterial count, *coliform* group and *Pseudomonas aeruginosa* did not show regular changes, and the microbial pollution degree in the samples varied in each month. The total bacterial count and *Pseudomonas aeruginosa* were detected in the samples in each month, but *coliform* group was not detected in the samples in August. The maximum detection rate of total bacterial count was 94.12% in September, and the lowest was 76.47% in August. The detection rate of *Pseudomonas aeruginosa* in the samples in June, September and November was the same, 11.11%, 11.76% and 12.50% respectively, which was the main pollution period. The highly polluted samples of *coliform* bacteria were in June and October, and the detection rates were 5.56% and 4.17% respectively. The results showed that

the samples in June were the main pollution time period, and the total number of *colonies*, *Coliforms* and *Pseudomonas aeruginosa* were all at a high pollution level. Compared between months, the total number of *colonies* (χ^2 = 5.75, p > 0.05), *coliform* group (χ^2 = 2.14, p > 0.05), *Pseudomonas aeruginosa* (χ^2 = 26.74, p > 0.05) there was no significant difference between the groups (**Table 2**).

Table 2. The positive rate of the microbe in terminal equipment fine drinking water from different month.

Month	*N*	Total bacterial count			*Coliform* group			*Pseudomonas aeruginosa*		
		Detection rate/%	Median/ (CFU/mL)	Maximum/ (CFU/mL)	Detection rate/%	Median/ (MPN/mL)	Maximum/ (MPN/mL)	Detection rate/%	Median/ (CFU/250 mL)	Maximum/ (CFU/250 mL)
6	36	83.33	102	10,000	5.56	ND	66	11.11	ND	140
7	40	77.50	59	14,000	2.50	ND	16	2.50	ND	16
8	34	76.47	54	12,000	0.00	ND	0	5.88	ND	46
9	34	94.12	50	11,000	2.94	ND	22	11.76	ND	10
10	48	85.42	121	660	4.17	ND	22	2.08	ND	10
11	40	87.50	33	12,000	2.50	ND	13	12.50	ND	50
Total	232	84.05	58	14,000	3.02	ND	66	7.33	ND	140

2.2. Microorganism exceeding standard in direct drinking water

According to the judgment standard, the overall over standard rate of 232 terminal direct drinking water was 9.48%, and the over standard rates of *Pseudomonas aeruginosa* and *coliform* were 7.33% and 3.02% respectively (**Figure 1**). Comparison between groups (χ^2 = 4.39, p < 0.05), the difference was statistically significant, indicating that *Pseudomonas aeruginosa* was the main pathogenic microorganism hazard factor.

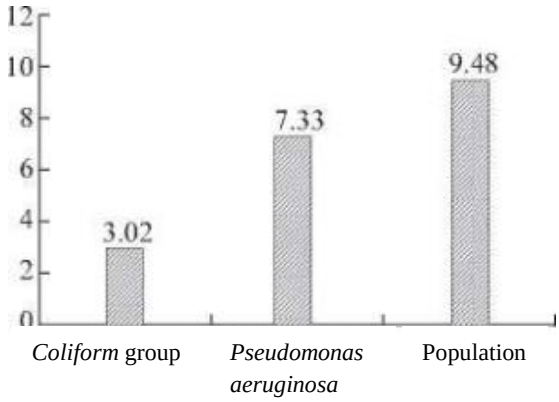

Figure 1. The rate of the microbe above the maximum limit in terminal equipment fine drinking water.

Considering the sample collection process, the over standard rate of terminal direct drinking water is family > School > community, which are 12.68%, 8.97% and 7.23% respectively. There is no significant difference between the groups (χ^2 = 1.36, p > 0.05), which may be related to the maintenance and repair of terminal direct drinking water equipment (**Table 3**). There is no consistency in the over standard rate of *Pseudomonas aeruginosa* and *coliform* in the sample collection process. *Pseudomonas aeruginosa* is the most over standard in the family sample, and the

school is the least over standard. The over standard rate of *coliform* is family > School > Community (**Table 3**).

Table 3. The rate of the microbe above the maximum limit in terminal equipment fine drinking water from various sources.

Total number of phase samples		Excess rate of *coliform* group/%	Exceeding standard rate of *Pseudomonas aeruginosa*/%	Total/%
Family	71	7.04	8.45	12.68
School	78	2.56	6.41	8.97
Community	83	0.00	7.23	7.23

Comparing the sample exceedance rate of each month, the exceedance rate from large to small is November > June > September > October > August > July, and there is no significant difference between the groups (χ^2 = 4.47, P > 0.05). The exceedance rates of *coliform* and *Pseudomonas aeruginosa* in the samples varied from month to month. The largest exceedance rate of *coliform* was in June, which was 5.56%; the maximum exceeding standard rate of *Pseudomonas aeruginosa* was 12.50% in November (**Table 4**). *Coliform* group (χ^2 = 2.14, p > 0.05), *Pseudomonas aeruginosa* (χ^2 = 6.74, p > 0.05) there was no significant difference between the groups in the month comparison of the over standard rate.

Table 4. Excessive microbial pollution in direct drinking water from terminals in different months.

Month	Total number of samples	Excess rate of *coliform* group/%	Exceeding standard rate of *Pseudomonas aeruginosa*/%	Total/%
June	36	5.56	11.11	13.89
July	40	2.50	2.50	5.00
August	34	0.00	5.88	5.88
September	34	2.94	11.76	11.76
October	48	4.17	2.08	6.25
November	40	2.50	12.50	15.00

2.3. Multiple pollution of direct drinking water

In 232 samples, there were multiple pollution exceeding the standard for 2 or more microbial indicators, and the total number of *colonies*, *coliform* group and *Pseudomonas aeruginosa* exceeded the standard at the same time by 0.86% (2/232); all of them come from family samples; the samples are from June and September respectively, which may be related to the replacement frequency of equipment accessories. The simultaneous pollution rate of total bacterial count and *coliform* group was 1.72% (4/232), and two samples were taken from home and school, respectively, in July, October and November. The total number of *colonies* and the simultaneous pollution rate of *Pseudomonas aeruginosa* were 6.47% (15/232), and the community > school > family were 2.59% (6/232), 2.16% (5/232) and 1.72% (4/232) respectively, which were found in all months.

3. Discussion

At present, direct drinking water is mainly divided into pipeline direct drinking water and terminal direct drinking water. Pipeline direct drinking water has certain installation limitations, while terminal direct drinking water is to connect the direct drinking machine directly with the tap water pipe and complete water treatment through the reverse osmosis membrane system in the drinking machine, which is more applicable and universal. It has become the mainstream drinking water method of direct drinking water for families, schools and communities [4,5,7], but its health quality is worrying. The average qualified rate of microbiological indicators of direct drinking water in Baotou City from 2009 to 2014 was 89.1%, mainly due to the excessive total number of *colonies* and *coliform* pollution [13]; the qualified rate of direct drinking water in Kaifeng area is 80.32%, and the total bacterial count is the main pollution exceeding standard factor [14]; the total qualified rate of water quality test results of piped direct drinking water in Haidian District of Beijing from 2014 to 2017 was 84.48%, and the unqualified items of microbial indicators were the total number of *colonies* and total *coliform* [15].

Among 232 samples monitored, the detection rate of total bacterial count was 84.05%, which was much higher than that of *coliform* and *Pseudomonas aeruginosa*. The total bacterial count was the main microbial pollution factor of direct drinking water, which was consistent with the relevant literature [13–15]. In view of the lack of sanitary standards for direct drinking water, the total number of *colonies* belongs to sanitary indicator bacteria, which will not affect public health in general, and excessive control of sanitary indicator bacteria and sterilization may lead to other health risks of direct drinking water [16], it is questionable to judge the quality of direct drinking water by the total number of *colonies*. Considering that GB 19298-2014 cancels the total number of *colonies*, it is determined that the quality of direct drinking water does not include the total number of *colonies*. The direct drinking water of families, communities and schools is polluted by the total number of *colonies* and *Pseudomonas aeruginosa*, while the *coliform* group is mainly detected in the samples of families and communities, while the samples of schools are not detected, indicating that the management and maintenance of school direct drinking water equipment are better than those of families and communities. According to the judgment standard, the overall over standard rate of 232 terminal direct drinking water was 9.48%, and the qualified rate was the same as that reported in relevant reports [13–15], mainly due to the over standard of *coliform* and *Pseudomonas aeruginosa*, which were 3.02% and 7.33% respectively. In the comparison of sample links, the highest rate of exceeding the standard of household direct drinking water is 12.68%, which is higher than 7.23% in the community and 8.97% in the school, which may be related to the untimely maintenance and repair of terminal direct drinking water equipment. Compared with the sampling months, the exceeding standard rate is 15.00%, 13.89%, 11.76%, 6.25%, 5.88% and 5.00% from November > June > September > October > August > July, respectively. There is no seasonal regular change trend, which is contrary to the reports of Chen Lei [9]. The monitoring results showed that the direct drinking water samples in each month and link were contaminated by *Pseudomonas aeruginosa*, with an overall pollution rate

of 7.33%, and the highest pollution rate in November was 12.50%. The pollution rate of household samples was higher than that of communities and schools. *Pseudomonas aeruginosa* is a common environmental microorganism, which is widely distributed. It is also a food borne opportunistic pathogen. It has strong resistance to adverse environment and can lead to acute enteritis, meningitis, sepsis, skin inflammation and other diseases. The pollution of *Pseudomonas aeruginosa* mainly comes from the production links [16,17]. The high pollution rate of *Pseudomonas aeruginosa* in terminal direct drinking water may be related to the failure to disinfect the activated carbon filter element of the equipment in time. There are multiple pollution samples of the total number of *colonies*, *coliform* bacteria and *Pseudomonas aeruginosa* in the family samples, and the rate of exceeding the standard is 0.86%, which are all collected in the family link. It is possible that the maintenance of the household terminal direct drinking water equipment is not carried out in accordance with the specifications.

The monitoring shows that the total bacterial count in the terminal direct drinking water is the main pollution factor, and the pollution of *coliform* and *Pseudomonas aeruginosa* exceeds the standard. It is suggested to carry out water quality monitoring of terminal direct drinking water, obtain basic data and promote the formulation of relevant standards. Government departments straighten out the main body of supervision and implement supervision and management; the operating enterprise shall strengthen the self-discipline of the industry, maintain the equipment and replace the components in strict accordance with the requirements, so as to ensure the good operation of the equipment.

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