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Assessment of the disinfection efficacy of hydrogen peroxide in neonatal bathing water pipelines

Min Liu[1], Richun Liu[1], Xiujuan Qu[1], Yuanli Chen[1*]

[1]Department of Hospital Infection Control, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, 519000, Guangdong, China

Abstract

Background *Pseudomonas aeruginosa* is a common conditional pathogen in newborn infant wash water, which can cause severe infections in neonates through direct contact or inhalation of aerosols. This study aimed to evaluate the disinfection efficacy of 0.4% hydrogen peroxide against *P. aeruginosa* contamination in newborn wash water and determine the optimal disinfection cycle.

Methods Pipelines were disinfected for 8 hours using a 0.4% hydrogen peroxide solution. Cold water was continuously monitored from five infant wash basins across four wards in the neonatal department for 12 weeks, with samples collected before disinfection, 1 day after disinfection, and weekly thereafter. The water samples were cultured at 35–37 °C for 48 hours, colony counts were recorded, and *P. aeruginosa* was identified using mass spectrometry. After determining the disinfection cycle, further monitoring and validation were conducted.

Results There was a 100% qualification rate in the first 3 weeks following the disinfection procedure. However, an obvious increase in the disqualification rate was observed in the fourth week. A second validation confirmed a similar trend, which indicates an elevated risk of recurring contamination after a 4-week disinfection cycle.

Conclusion The 8-hour soaking method using 0.4% hydrogen peroxide demonstrated short-term efficacy and was suitable for high-frequency disinfection of newborn wash water. This study provides scientific evidence for the prevention and control of *P. aeruginosa* contamination in neonatal wash water, and emphasizes the importance of multidimensional interventions.

Keywords: *Pseudomonas aeruginosa*, neonatal department, hydrogen peroxide disinfection, water quality monitoring, disinfection cycle

***Correspondence:**
Yuanli Chen; Email: chenyl1990@126.com

Background

Pseudomonas aeruginosa is a widely distributed opportunistic pathogen, which is highly invasive in newborns with immature immune systems [1,2]. It can cause severe multi-system infections, including skin and mucosal damage, respiratory tract infections, sepsis, multiple organ failure, and even death [3]. Its pathogenic mechanism is closely related to infection routes; thus, the prevention and control of hospital-acquired infections requires multi-dimensional interventions together with environmental management [4]. *P. aeruginosa* can directly contact the skin or umbilical cord stump of newborns through water, which leads to local infections characterized by skin redness and swelling, pyogenic rash, omphalitis, and even ulcers [5]. Without timely treatment, the infection can spread to subcutaneous tissues or the blood and cause systemic infection. In severe cases, it may be complicated with disseminated intravascular coagulation, shock, and multiple organ failure, with a mortality rate of 20% – 50% [6]. In addition, *P. aeruginosa* contamination in newborn bathing water is associated with respiratory tract damage in newborns. The impact of bathing water flow or defects in drainage system design (such as traps) can cause the rupture of *P. aeruginosa* biofilms and release aerosols containing bacteria [7]. Owing to the immature respiratory system of newborns, *P. aeruginosa* aerosols may invade the body through the nasal cavity or oral cavity. After inhalation, *P. aeruginosa* can colonize the respiratory tract or spread to the lungs, which can result in pneumonia [8]. Excessive *P. aeruginosa* in newborn bathing water poses a serious threat to newborns, which necessitates multi-dimensional prevention and control measures, including water source control, disinfection technology, and process management to reduce infection risks and safeguard the health and safety of newborns [9].

From the fourth quarter of 2023 to the third quarter of 2024, *P. aeruginosa* was detected in the bathing water of some newborns in the Neonatology Department of our hospital. Subsequent measures were taken, including slow water discharge for 1 hour every day, switching from an anti-scald mode to a high-temperature sterilization mode, integrating electric water heaters of bathing pools into the isolation rooms, disassembling and cleaning/disinfecting the filter membranes of all faucets, and disinfecting the bathing pools with 500 mg/L chlorine-containing disinfectant. However, *P. aeruginosa* contamination in the newborn bathing water was not completely controlled. Monitoring data in the fourth quarter of 2024 showed that *P. aeruginosa* was cultured from the cold water of five bathing pools, which attracted great attention. Following literature research and expert consultation, the water from the water pipelines in the Neonatology Department was completely drained, and food-grade hydrogen peroxide (greater than 0.4%) was injected and allowed to soak and disinfect the pipelines for 8 hours, followed by 12 consecutive weeks of water culture monitoring to evaluate the disinfection effect, determine the disinfection cycle, and ensure the water safety of newborns.

Materials and Methods

Sampling Areas and Frequency

Cold water samples were collected and tested from five newborn bathing pools, including two in the general ward, and one each in the intensive care ward, transitional ward, and isolation ward of the Neonatology Department. Sampling and monitoring were conducted before disinfection, 1 day after disinfection, and weekly for 12 consecutive weeks. The disinfection cycle was determined based on the tracking results, and re-sampling was performed for verification. The first-round disinfection period was between

December 30, 2024, and February 24, 2025 (84 days), with 12 rounds of sampling and monitoring. The second-round disinfection period was between April 14, 2025, to May 12, 2025, (28 days) with four rounds of sampling and monitoring.

Disinfectant Concentration and Disinfection Method

Disinfectant solution was prepared by adding one bottle of Nuofu NW-100 stock solution (hydrogen peroxide content, 45%–50%; silver ion content, 0.042%–0.05%; Figure 1a) to 100 L of water according to the product instructions. After draining the water in the ward pipelines, the disinfectant was injected using a pressure pump (Delixi Whole-house Booster Self-priming Pump). Detection was carried out at the terminal faucet of each bathing pool to ensure that all faucets were soaked in the disinfectant. Each faucet was regarded as qualified if the hydrogen peroxide concentration was over 0.4% (Figure 1b). Disinfection was performed from 16:00 to 24:00 for an 8-hour immersion. After disinfection, the residual disinfectant solution was drained into a pool to disinfect the surfaces of the infant bathing pool, and then drained into the sewer. The low concentration of the preparation ensures that it is safe for use, with limited pipe corrosion and no residue.

Sampling Method

Before sampling, water was run for 15 minutes. During sampling, aseptic operation principles were strictly followed. Fifty milliliters of water samples were collected using sterile sampling utensils.

Detection Method

The culture method was adopted for detection. After shaking and thoroughly mixing the collected water samples, 1 mL of the sample was inoculated onto nutrient agar medium (Guangzhou Dijing LS0309). The medium was incubated at 35–37 °C for 48 hours. Subsequently, the number of colonies on the plate was counted, and mass spectrometry was conducted to identify *P. aeruginosa* using the VITEK® MS microbial mass spectrometry identification system.

Qualification Standard for Monitoring

The following document was followed for water monitoring: *Standards for Drinking Water Quality* (GB 5749-2022)

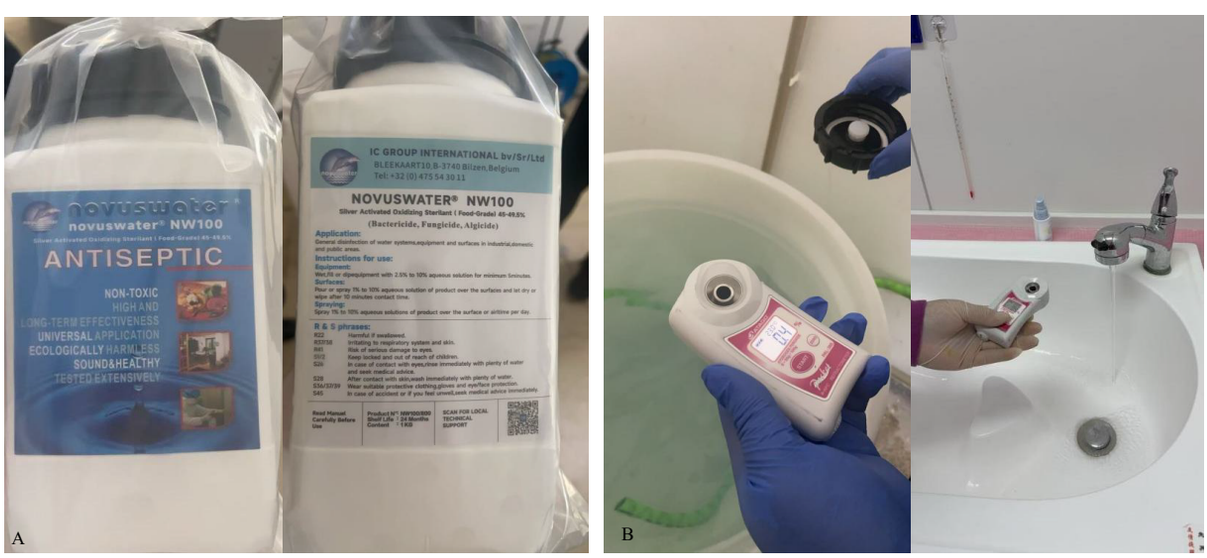

Figure 1. Disinfectant and Terminal Detection. A: Hydrogen peroxide stock solution; B: terminal detection results.

Results

Overview of Overall Trend

The monitoring results showed a significant downward trend in the initial stage of disinfection (Weeks 1–4). The qualification rate of water culture reached 100% from Week 1 to Week 3. However, the data of some monitoring sites gradually showed an upward trend starting from Week 4 (Figure 2). Therefore, the initial disinfection cycle was determined to be 4 weeks.

Verification of the Disinfection Cycle

To verify the reliability of the 4-week disinfection cycle, a second pipeline disinfection was conducted, followed by detection every 2 weeks. The detection values of all monitoring sites were 0 on the first day after disinfection, with an upward trend from Week 2 to Week 3, and some monitoring sites exceeded the standard at Week 4 (Figure 3). The comparison of the qualification rates between the first and second pipeline disinfections is shown in Table 1.

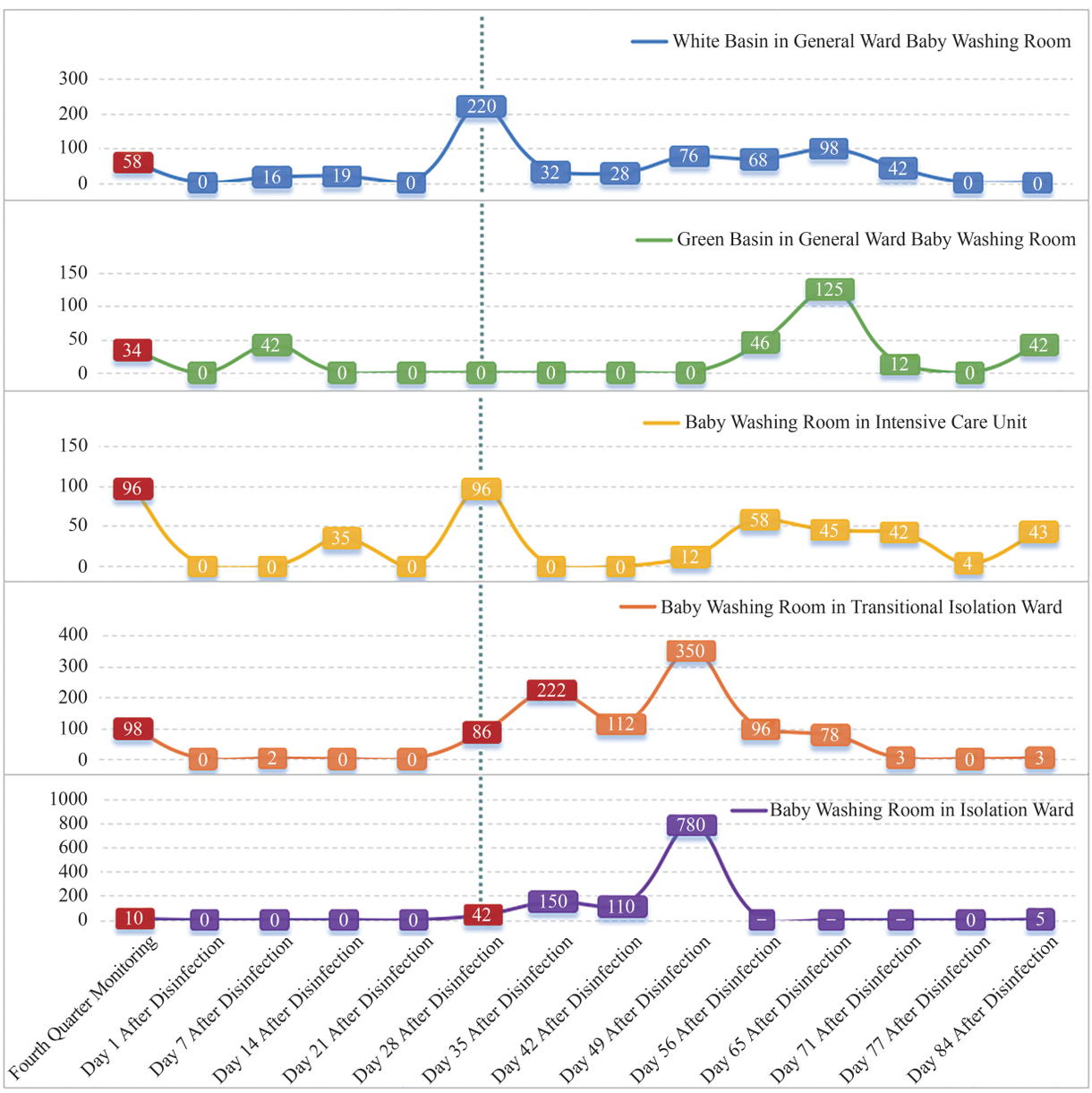

Figure 2. Water Culture Monitoring Results of Each Sampling Site Before and After Disinfection. Red indicates the presence of *P. aeruginosa* in the culture.

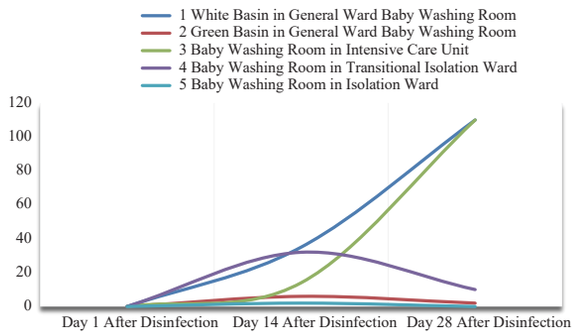

Figure 3. Water culture monitoring results after the second disinfection.

Table 1. Comparison of qualification status of newborn bathing water after the first and second disinfections

	First Disinfection	Second Disinfection
Day 1 after disinfection	100%	100%
Day 14 after disinfection	100%	100%
Day 28 after disinfection	40%	60%

Discussion

As an opportunistic pathogen, *P. aeruginosa* is highly invasive to newborns since this group of individuals is immunocompromised. It can cause local infections (such as omphalitis and pyoderma) through direct contact between bathing water and the newborns' skin or umbilical cord stumps, and even lead to pneumonia or systemic infections through the inhalation of aerosols. This highlights the urgency to prevent and control *P. aeruginosa* contamination in newborn bathing water [10–12] through multi-dimensional interventions, including water source control, disinfection technology, and process management. In particular, strict management of bathing water in high-risk areas of the neonatology department (such as isolation wards and intensive care wards) is essential to control infection risks and reduce infection rates [13,14].

This study demonstrated that disinfection using an 8-hour immersion with 0.4% hydrogen peroxide solution significantly reduced *P. aeruginosa* contamination in the short term (Weeks 1–3), with a 100% qualification rate on the first day after disinfection. This finding is consistent with the high-efficiency bactericidal properties of hydrogen peroxide in a similar study [15]. However, the number of *P. aeruginosa* colonies in some monitoring sites increased after Week 4, which indicates that the disinfection effect is time-limited. It is inferred that the disinfection effect can last for 4 weeks, with limitations in the long run. A rebound in contamination may occur 4 weeks after a single disinfection, which might be related to biofilm formation in pipelines, regeneration of residual strains, or cross-contamination from the environment [16]. Furthermore, operational factors may contribute to a recurrence in contamination; for example, insufficient water discharge time (less than 15 minutes) before sampling may not completely remove residual bacteria. In subsequent work, attention should be paid to pipeline maintenance and post-disinfection flushing procedures, such as extension of the water discharge time to 30 minutes.

This study identified an initial 4-week disinfection cycle through two phases of monitoring. In the first verification, the qualification rate remained at 100% between Weeks 1 and 3 after disinfection, although some sites exceeded the standard at Week 4 (Figure 2), which suggests that Week 4 may be a critical threshold. In the second verification, the qualification rate remained at 100% between Weeks 2 and 3 after re-disinfection, although contamination reappeared at Week 4 (Figure 3), which confirms that the risk of recurring contamination is relatively high after the 4-week cycle.

Although this study provides preliminary evidence of the effectiveness of hydrogen peroxide in pipeline disinfection, there are some limitations. First, the sample scope only covered five bathing pools in four wards (with other areas of the neonatology department untested), which may limit the extrapolation of the results. Secondly, a single intervention measure was

used, with no comparison with other disinfectants (such as sodium hypochlorite) or combined disinfection protocols. Thirdly, there is a relatively insufficient sample size, which directly affects the rigor of the research methodology and reliability of the conclusions. Owing to the limited sample size, we adopted the pass rate as the primary evaluation indicator. While this method can provide a preliminary assessment of effectiveness, it lacks strict statistical validation. Hence, the results may not fully reflect the broader population, and caution should be exercised to avoid presuming that the conclusions apply to other contexts. Nevertheless, this study provides valuable preliminary references and basic data for practices in relevant fields, and lays a foundation for in-depth research. We suggest that future studies can validate and refine the results of this study through expansion of the sample size and adoption of more rigorous statistical analysis methods.

Finally, the current detection method using conventional nutrient agar culture has multiple drawbacks. On the one hand, it is time-consuming, which may delay early warnings of contamination. On the other hand, the nutrient-rich medium has a low detection rate for oligotrophic bacteria commonly found in the environment (such as some difficult-to-culture microorganisms like *Pseudomonas* and *Acinetobacter*). It cannot fully reflect the actual microbial community structure of pipeline contamination, and excessive nutrients inhibit the growth of oligotrophic bacteria, which increases the risk of missed detection. The main reason why the R2A low-nutrient medium was not adopted in this study is that early stage of the experiment focused on rapid screening of common pathogenic bacteria, with limited understanding of the potential risks of oligotrophic bacteria in the pipeline environment of the neonatology department. In the future, R2A medium combined with molecular biological methods can be introduced to achieve a faster and more comprehensive assessment of contamination.

The results of this study can directly guide clinical practice, strengthen the monitoring system, and establish a dynamic water quality monitoring mechanism, with more frequent testing implemented in high-risk areas such as isolation wards and intensive care wards.

In addition, it is necessary to strengthen multidisciplinary collaboration and unite the hospital infection control department, microbiology laboratory, and logistics department to formulate standardized operating procedures (SOPs) for disinfection and ensure that disinfection measures are fully implemented. Scientific prevention and control should be adopted to reduce the risk of *P. aeruginosa* contamination, safeguard the safety of newborn bathing water, and decrease the incidence of related infections.

Conclusion

This study evaluated the application of hydrogen peroxide disinfectant in newborn bathing water from the neonatology department by monitoring and analyzing *P. aeruginosa* contamination and exploring the rationality of the disinfection cycle. The 8-hour disinfection protocol using an 0.4% hydrogen peroxide solution exhibits short-term efficacy and is suitable for high-frequency disinfection scenarios of newborn bathing water. These research results will help prevent and control hospital-acquired infections in newborns.

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Ethics approval

The research protocol was reviewed by the Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-sen University. Data collection is sourced from public resources and does not require ethical approval.

Author contributions

Y. L. Chen was responsible for the conception and design. M. Liu drafted the initial manuscript. R.C. Liu analyzed the data. X.J. Qu, M. Liu and R.C. Liu were responsible for data collection. All authors have read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no competing interests.

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