

A study on detection of waterborne pathogens in hospital water systems for prevention of healthcare-associated infections: A prospective cross-sectional observational study.

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Abstract

Background

Hospital water systems can act as reservoirs of opportunistic waterborne pathogens. Biofilm formation within pipelines, taps and distal outlets promotes microbial persistence and increases healthcare-associated infection risk.

Objectives

To detect waterborne pathogens in hospital water systems and assess their relevance to healthcare-associated infection prevention.

Methods

This prospective cross-sectional observational study was conducted over 12 months in a tertiary care teaching hospital. Ninety environmental samples were examined: 35 tap water samples, 35 tap swabs, 5 reverse osmosis water samples, 10 air-conditioning water samples and 5 drinking water samples from intensive care units, wards, operation theatres, laboratories, dialysis areas and water outlets. Samples were processed using standard microbiological methods with Blood agar, MacConkey agar and Buffered Charcoal Yeast Extract agar. Isolates were identified by colony morphology, Gram staining and biochemical tests. Data were summarized using frequencies, percentages and a 95% confidence interval for culture positivity.

Results

Bacterial growth was detected in 19 of 90 samples, giving an overall contamination rate of 21.1% (95% CI: 14.0%-30.6%). Tap water had the highest positivity at 31.4%, followed by tap swabs at 22.9%. All isolates were Gram-negative bacilli. *Pseudomonas aeruginosa* predominated, followed by *Klebsiella pneumoniae*, *Escherichia coli* and *Citrobacter freundii*. *Legionella pneumophila* was not isolated. Reverse osmosis, air-conditioning and drinking water samples showed no growth. Contamination was concentrated at distal tap outlets, identifying potential environmental reservoirs.

Conclusion

Hospital water outlets, especially taps, were important environmental points for microbial contamination. Routine surveillance, timely maintenance and point-of-use infection control measures are essential to reduce water-associated infection risk.

Recommendations

Hospitals should implement structured water-safety monitoring, periodic flushing, tap outlet disinfection, documentation of corrective actions and integration of water surveillance with infection control audits.

Keywords: Healthcare-associated infections; Hospital water systems; *Pseudomonas aeruginosa*; Biofilm; Waterborne pathogens; Microbiological surveillance

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Introduction

Healthcare-associated infections remain a major concern for patient safety because they increase morbidity, prolong hospital stay and add avoidable costs to care delivery [1]. Although direct contact, contaminated hands and medical devices are common transmission routes, the hospital environment also deserves close attention. Water systems are of special importance because they are used continuously in patient-care areas and are connected with hand hygiene, cleaning, respiratory care, dialysis, wound care and routine clinical services [2,3]. In large hospitals, water distribution networks contain storage tanks, long pipelines, valves, faucets, showers and terminal outlets. These structures create multiple ecological niches where organisms persist even when the incoming water appears clean.

The biological risk from hospital water is closely linked to biofilm. Biofilms are structured microbial communities attached to wet surfaces, embedded in a protective matrix and capable of releasing organisms intermittently into flowing water [4]. Stagnation, corrosion, warm temperature zones, irregular flow and residual organic matter promote biofilm development in premise plumbing. Once established, biofilms reduce the effectiveness of routine disinfection and create a protected reservoir for opportunistic pathogens. Organisms such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, nontuberculous mycobacteria and *Legionella pneumophila* have been associated with healthcare water systems and water-related devices [5-7].

Pseudomonas aeruginosa is particularly relevant in hospital water surveillance because it survives well in moist environments, colonizes taps and sinks, forms persistent biofilm and exhibits intrinsic or acquired antimicrobial resistance [8,9]. Previous investigations have linked contaminated water outlets and wastewater systems with colonization or infection in intensive care, haematology, neonatal and other high-risk units [7,10,11]. *Legionella pneumophila* is another important waterborne pathogen because it can proliferate in warm water networks and infect susceptible patients through inhaled aerosols [12,13]. The detection of these organisms has implications for environmental monitoring, engineering maintenance, patient safety and antimicrobial stewardship.

In resource-limited settings, routine microbiological surveillance of hospital water systems is not always systematic. Culture-based monitoring remains a practical method for identifying contamination and planning corrective measures in healthcare facilities. Local data are needed because contamination patterns vary according to water source, plumbing design, outlet maintenance, disinfection practice and patient-care intensity

[14]. The present study was conducted to isolate and identify waterborne microorganisms from selected hospital water outlets and tap surfaces, determine the prevalence of microbial contamination, describe the distribution of organisms across sample types, identify the predominant bacterial species and emphasize the role of regular water surveillance in preventing healthcare-associated infections.

Materials and methods

Study design

This study was designed as a prospective cross-sectional observational environmental microbiological surveillance study conducted over a 12-month period.

Study setting

The study was conducted at Government General Hospital (GGH), Suryapet, Telangana, India, a tertiary care teaching hospital, and was coordinated through the Department of Microbiology. Environmental sampling covered intensive care units, neonatal intensive care units, operation theatres, dialysis areas, wards, laboratories, reverse osmosis outlets and drinking water points, with microbiological processing performed in the hospital microbiology laboratory. The study was conducted from January to December 2024

Study population and sample size

The study population consisted of environmental samples obtained from active water outlets and tap surfaces within the hospital. Ninety samples were included, comprising 35 tap water samples, 35 tap swab samples, 5 reverse osmosis water samples, 10 air-conditioning water samples and 5 drinking water samples. The sample size was based on accessible surveillance points during the study period and included both patient-care and utility sites to provide a practical assessment of hospital water contamination.

Eligibility criteria

Water samples from active outlets used for patient care, staff use or drinking purposes were included. Tap swabs from frequently used outlets and samples from high-risk areas such as intensive care units, neonatal intensive care units and operation theatres were also included. Samples from toilets, drains, non-clinical areas, leaking containers, inadequately labelled specimens, insufficient volume or delayed transport were excluded.

Sample collection

Approximately 10 mL of water was collected aseptically in sterile, leak-proof containers from each selected site. Where applicable, samples were collected before and after flushing to assess the effect of stagnation. Tap swabs were collected using sterile cotton swabs moistened with sterile normal saline and rotated over the inner tap surface to sample biofilm-prone areas. All specimens were labelled with source, date and sample type and transported promptly to the microbiology laboratory.

Microbiological processing and identification

Water samples were screened for possible fecal contamination using the hydrogen sulfide test. Water and tap swab specimens were inoculated onto Blood agar, MacConkey agar and Buffered Charcoal Yeast Extract agar using standard microbiological precautions [7,8]. Plates were incubated aerobically at 37°C and examined at 24 and 48 hours. Buffered Charcoal Yeast Extract plates were observed for 5–7 days for *Legionella* recovery [12,13]. Colony morphology, pigmentation, lactose fermentation and hemolysis were recorded. Gram staining and biochemical tests, including oxidase, catalase, indole, citrate, urease and Triple Sugar Iron reactions, were used for species-level identification wherever feasible [9].

Quality Assurance

Strict aseptic precautions were followed during sample collection and processing to minimize external contamination. Culture media were checked for sterility and performance before use. Known control strains were used wherever applicable to ensure reliability of microbiological procedures. A uniform processing protocol was followed for all samples to reduce measurement bias.

Bias

Potential selection bias was recognized because sampling was limited to accessible surveillance points within a single hospital. This was minimized by including both high-risk and routine-use clinical areas and by applying the same eligibility criteria across sample categories. Sampling contamination bias was reduced through aseptic collection, sterile leak-proof containers, prompt transport and standardized tap-swab procedures. Measurement

and laboratory-processing bias were minimized by using a uniform culture protocol, defined incubation conditions, sterility and performance checks for culture media, appropriate control strains where applicable, and consistent organism-identification criteria. Because patient isolates were not linked to environmental isolates, the findings were interpreted as evidence of environmental contamination and potential infection-prevention relevance rather than proof of water-to-patient transmission.

Data Analysis

Data were recorded according to sample type, sampling source, culture positivity, and organism isolated. The prevalence of water contamination was calculated as the proportion of culture-positive samples among the total number of samples processed. Results were summarized using frequencies and percentages. The overall culture-positivity proportion was additionally reported with a 95% confidence interval calculated using the Wilson score method. Because the study was designed as descriptive environmental surveillance and did not test a prespecified comparative hypothesis, no inferential group-comparison test was applied.

Ethical Considerations

Institutional approval was obtained from Government Medical College, Suryapet, Telangana, India, before initiation of the study. As the study involved only environmental water samples and did not include human participants, patient data, or biological specimens, informed consent was not required.

Results

A total of 90 samples were analyzed, comprising 35 tap water samples, 35 tap swab samples, 5 reverse osmosis water samples, 10 air-conditioning water samples and 5 drinking water samples collected from hospital water outlets and related environmental points. Overall, 19 samples showed bacterial growth, giving a contamination rate of 21.1%. Culture positivity differed across sample categories, with tap water showing the highest positivity, followed by tap swabs. No bacterial growth was detected from reverse osmosis water, air-conditioning water or drinking water samples, as shown in Table 1. The overall culture-positivity proportion of 21.1% had a 95% confidence interval of 14.0%–30.6%.

Table 1: *Distribution of samples and culture positivity*

Sample type	Total samples, n	Positive samples, n	Positivity rate, %
Tap swab	35	8	22.9
Tap water	35	11	31.4
Reverse osmosis water	5	0	0.0
Air-conditioning water	10	0	0.0
Drinking water	5	0	0.0
Total	90	19	21.1

Among the five sample categories, tap water accounted for 11 positive samples with a positivity rate of 31.4%. Tap swabs accounted for eight positive samples with a positivity rate of 22.9%. These findings indicate that distal water outlets and faucet surfaces were the principal contaminated sites in the sampled hospital environment.

The organism profile showed exclusive recovery of Gram-negative bacilli. *Pseudomonas aeruginosa* was the most frequent isolate, accounting for 42.1% of the total isolates. *Klebsiella pneumoniae* accounted for 31.6%, *Escherichia coli* for 21.1% and *Citrobacter freundii* for 5.3%, as summarized in Table 2.

Table 2: *Distribution of isolated organisms*

Organism	Number of isolates (n=19)	Percentage, %
<i>Pseudomonas aeruginosa</i>	8	42.1
<i>Klebsiella pneumoniae</i>	6	31.6
<i>Escherichia coli</i>	4	21.1
<i>Citrobacter freundii</i>	1	5.3

When only culture-positive samples were considered, tap water contributed 57.9% of all positive results, whereas tap swabs contributed 42.1%. This source-wise pattern, shown in Table 3,

supports the interpretation that both water from the outlet and the inner tap surface contributed to the contamination burden.

Table 3: *Source-wise contribution among positive samples*

Source type	Total samples, n	Positive samples, n	Contribution among positives, %
Tap swabs	35	8	42.1
Tap water	35	11	57.9

The key findings are summarized in Table 4. *Legionella pneumophila* was not isolated from any sample. The absence of growth from reverse osmosis and drinking water points indicates

satisfactory microbiological performance of these treated water systems during the sampling period.

Table 4: *Key microbiological findings*

Parameter	Observation
Total samples analyzed	90
Total culture-positive samples	19 (21.1%)
Most contaminated source	Tap water
Predominant organism	<i>Pseudomonas aeruginosa</i>
<i>Legionella pneumophila</i> detection	Not detected
Dominant bacterial group	Gram-negative bacilli

Discussion

The present study identified bacterial contamination in 21.1% of hospital environmental water-related samples, indicating that hospital water systems can serve as reservoirs for opportunistic pathogens. This finding is clinically important because water exposures in healthcare facilities occur repeatedly during routine patient care, cleaning and equipment handling [2,3]. Previous reviews have shown that contaminated water reservoirs have been linked with outbreaks involving Gram-negative bacilli, *Legionella* species and other opportunistic pathogens, particularly in vulnerable patients [2,6]. The observed contamination rate highlights the need to treat hospital water surveillance as an active infection prevention activity rather than a maintenance-only function. It also supports early corrective action before environmental contamination becomes clinically significant.

Tap water showed the highest positivity, and tap swabs also yielded bacterial growth. This pattern supports the role of distal outlets as important colonization sites. Faucets, aerators and inner outlet surfaces create wet interfaces where biofilm develops and intermittently releases organisms into water flow [4,7]. Earlier studies on healthcare water systems reported that contamination is often concentrated at distal ends and can persist despite routine cleaning [7,8]. The absence of growth from reverse osmosis and drinking water samples suggests that treated water systems were functioning effectively during sampling, although continuous monitoring remains necessary because water quality changes with maintenance, storage and outlet use.

Gram-negative bacilli predominated in the present study, with *Pseudomonas aeruginosa* as the leading isolate. This finding is consistent with the known ecology of *P. aeruginosa*, which survives in moist hospital environments, forms biofilm and adapts to adverse conditions [8,9]. Waterborne transmission of *P. aeruginosa* has been documented in intensive care and haematology settings, and interventions aimed at contaminated outlets have reduced clinical isolates in some reports [10,11]. The recovery of *Klebsiella pneumoniae* and *Escherichia coli* further indicates that water and tap surfaces can harbour organisms with recognized healthcare relevance, including potential antimicrobial-resistant strains [5,14].

Legionella pneumophila was not detected in this study. This result is reassuring but should be interpreted with caution because *Legionella* recovery depends on temperature, residual disinfectant, sample volume, culture conditions and the presence of viable but non-culturable forms [12,13]. Culture-based methods are practical for routine surveillance, but molecular methods improve detection when *Legionella* risk is suspected or when high-risk units report unexplained pneumonia clusters. Overall, the study reinforces the value of scheduled water sampling, prompt reporting, outlet disinfection, flushing protocols, plumbing maintenance and coordination between microbiology, engineering and infection control teams [2,6,13].

Generalizability

The findings are most applicable to tertiary care hospitals with comparable water distribution networks, patient-care areas and

conventional culture-based microbiology facilities. The emphasis on tap water and tap swabs is relevant to settings where distal outlets are frequently used for hand hygiene, cleaning and patient-care support. Generalization to hospitals with different plumbing age, disinfection systems, water source and climate requires local surveillance because environmental contamination patterns vary considerably across similar institutions.

Conclusion

This prospective cross-sectional observational study demonstrated that 21.1% of hospital water-related environmental samples were culture positive. Contamination was concentrated in tap water and tap swabs, confirming the importance of distal outlets as potential reservoirs for opportunistic pathogens. Gram-negative bacilli were the only isolates recovered, and *Pseudomonas aeruginosa* was the predominant organism, followed by *Klebsiella pneumoniae*, *Escherichia coli* and *Citrobacter freundii*. *Legionella pneumophila* was not detected, and treated water sources showed no growth. These findings support regular microbiological surveillance, timely outlet maintenance, flushing, disinfection and infection control review as practical steps to reduce the risk of water-associated healthcare infections and guide early remedial action before patient exposure occurs in hospital settings.

Limitations

The study was limited to 90 environmental samples from a single tertiary care hospital. Molecular confirmation and antimicrobial susceptibility testing were not performed. Seasonal variation, residual disinfectant levels, water temperature and detailed plumbing characteristics were not assessed. Patient isolates were not compared with environmental isolates, limiting direct attribution of contamination to healthcare-associated infections. Quantitative bacterial load estimation was also unavailable.

Recommendations

Hospitals should establish a written water safety and surveillance plan covering high-risk units, routine-use wards and treated water outlets. Tap water and inner faucet surfaces should be sampled periodically because these sites showed the greatest contamination. Engineering teams should document flushing, descaling, aerator cleaning, outlet replacement and disinfection activities. Microbiology laboratories should report significant isolates rapidly to the infection control committee. Antimicrobial susceptibility testing and molecular methods should be added when outbreaks, resistant organisms or *Legionella* risk are suspected. Regular staff training is needed to prevent unsafe use of tap water for patient-care procedures and to strengthen environmental safety practices.

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Abbreviations

BCYE: Buffered Charcoal Yeast Extract;
GGH: Government General Hospital;
H₂S: Hydrogen sulfide;
TSI: Triple Sugar Iron.

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The study had no funding.

Conflict of interest

The authors declare no conflict of interest.

Author Contributions

Dr Bidanchi Ch Marak contributed to study conception, sample collection, microbiological processing, data recording, literature review, and drafting of the manuscript.

Dr S Ruth Ramya contributed to study design, supervision of laboratory procedures, data interpretation, manuscript preparation, and critical revision of the intellectual content.

Dr Aditi Tomer Thakur contributed to microbiological methodology, quality assurance, data analysis, literature review, and revision of the manuscript.

Dr Taruni contributed to study conception, sample collection, literature review, and drafting of the manuscript.

Dr R Kondal Rao provided overall academic supervision, guidance in study planning, interpretation of findings, critical review of the manuscript

Dr Nagasaikaran Sade provided literature review, data analysis and drafting of the manuscript.

Data availability

Data available on request

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