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Microbiological Assessment of Biomedical Waste, Environmental Monitoring and Sterilization Effectiveness with Antibiotic Profile in a Specialized Eye Hospital of Dhaka City, Bangladesh

Fazra Jahan Simthy[1], Maharun Nesa Haimu[1], Famiya Ahmad[1], Israt Jahan Nuhi[1], Ayesha Binte Shamim[2], Md Aftab Uddin[1] and Sifat Uz Zaman[1]*

[1]*Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka, Bangladesh*

[2]*Department of Microbiology, Gono Bishwabidyalay, Savar, Dhaka, Bangladesh*

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Hospital environments, biomedical waste, and water sources can act as potential reservoirs for microorganisms that contribute to healthcare-associated infections (HAIs). Ensuring effective sterilization, water safety, and environmental hygiene is therefore crucial for infection prevention and patient safety. This study aimed to assess the microbiological presence of hospital drinking water, biomedical waste, and operating theatre (OT) environments, as well as to evaluate sterilization effectiveness and antibiotic susceptibility patterns of the isolated microorganisms. Samples were collected from hospital drinking water, biomedical waste bins, and OT surfaces, including beds, floors, and trays. Microorganisms were isolated and identified by standard microbiological methods using Nutrient Agar and different types of selective and differential media, along with Gram staining. Antibiotic susceptibility was determined by the Kirby-Bauer disc diffusion method. Sterilization performance was evaluated over 17 autoclave cycles using both Class N and Class B autoclaves. Physical, chemical (Bowie-Dick test, Class 5 integrator, and autoclave tape), and biological indicators containing *Geobacillus stearothermophilus* spores were employed for process validation. *Escherichia coli* was isolated from hospital drinking water, *Klebsiella* spp. from biomedical waste, and *Staphylococcus aureus* from OT surfaces. *E. coli* showed complete sensitivity to all tested antibiotics, whereas *Klebsiella* spp. displayed resistance to Cefuroxime but remained sensitive to other antibiotics. *Staphylococcus aureus*, identified as a normal environmental flora, was found on OT beds and floors that were regularly disinfected with formaldehyde. Sterilization tests confirmed 100% effectiveness across all autoclave cycles, indicating optimal sterilization performance and reliability of all indicator systems. Overall, the findings emphasize that rigorous sterilization procedures, proper biomedical waste management, and regular environmental disinfection substantially minimize microbial contamination in healthcare facilities. Continuous microbiological monitoring and antibiotic susceptibility testing are essential to detect resistant organisms early and sustain a safe, infection-free hospital environment.

Keywords: Biomedical waste (BMW), Environmental monitoring, Sterilization effectiveness, Antibiotic susceptibility, Infection control, Healthcare-associated infections (HAIs)

INTRODUCTION

Biomedical waste refers to any waste that is produced during healthcare-related activities such as diagnosis, treatment, immunization, or research involving humans or animals. It includes materials like used syringes, bandages, laboratory samples, body fluids, expired medicines, and other hospital items that may contain infectious or hazardous substances (1). Biomedical waste (BMW) poses a serious threat to community health because it often carries infectious microorganisms that can spread diseases if not handled or disposed of properly (2). Even healthcare facilities with a narrow focus, routinely produce a variety of biomedical waste materials. These include contaminated syringes, cotton, gauze, and other items that come in contact with patients. Such wastes can act as potential sources of pathogenic microorganisms, including Gram-positive bacteria such as *Staphylococcus aureus* and *Enterococcus* spp. Gram-negative organisms like *Escherichia coli*, *Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*, as well as opportunistic fungi such as *Candida* spp. In some situations, viral pathogens may also be present (3). Biomedical waste management involves safe treatment and disposal practices to minimize infection risks and environmental pollution. Autoclaving is one of the most effective non-incineration methods, where high-pressure steam at 121–134°C sterilizes infectious materials such as contaminated dressings, glassware, and microbiological cultures (4, 5). Sharps, especially needles, pose serious hazards of injury and disease transmission; therefore, needle destroyers are commonly used to cut or melt needle tips, preventing reuse and reducing needle-stick injuries (6). Proper segregation using color-coded bins ensures waste is treated correctly: yellow, red, and green bins can be employed according to the waste type—for example, infectious wastes are placed in the red bin (7, 8). Hospital environments can

***Corresponding Author**: Sifat Uz Zaman, Assistant Professor, Department of Microbiology, Stamford University Bangladesh; Email: sifat.zaman9@gmail.com; Contact: +880-162-1970240*

act as reservoirs for infectious microorganisms, which may survive on surfaces, medical instruments, ventilation systems, and even within water networks. Commonly encountered pathogens include *Staphylococcus aureus, Pseudomonas* spp., *Acinetobacter* spp., and fungal organisms such as *Aspergillus;* these microbes may contribute to healthcare-associated infections (HAIs), leading to conditions such as surgical site infections, eye infections, bloodstream infections, and other hospital-acquired complications (9). The environment of the operation theatre (OT) holds particular importance in infection control. The OT is a critical area where sterility must be maintained, yet it is also highly vulnerable to microbial contamination. Regular environmental monitoring of the OT includes swab sample collection from surfaces such as beds, floors, and instrument trays. These samples help identify hidden reservoirs of pathogens and ensure that cleaning and disinfection procedures are effective (10, 11). By routinely checking the microbiological status of the OT environment, hospitals can minimize the risk of surgical site infections and improve overall patient safety (12).

Sterilization plays a vital role in both healthcare facilities and microbiology laboratories because it eliminates all forms of microorganisms, including those that are highly resistant. By ensuring that medical instruments, laboratory tools, surfaces, and biomedical waste are completely free from pathogens, sterilization helps maintain patient safety and prevents the spread of infections. When this process is not carried out effectively, even a few surviving microbes can create serious problems, including hospital-acquired infections in patients, contamination of laboratory experiments, or the unintentional release of pathogens into the surrounding environment (13). To ensure effectiveness, sterilization processes are routinely tested using different types of indicators. Physical indicators (such as gauges and digital displays) confirm that the autoclave has reached the required temperature, pressure, and time. Chemical indicators (like autoclave tapes or strips) change color when exposed to appropriate sterilization conditions, providing quick visual confirmation. Biological indicators, considered the gold standard, use heat-resistant spores of *Geobacillus stearothermophilus* to verify that even the most resistant organisms are destroyed (14). Regular use of these indicators ensures that sterilization is not just assumed but scientifically validated. The quality of water used in autoclaving also plays an important role. Reverse osmosis (RO) water is preferred because it is free from minerals and impurities that may cause scaling or residue buildup inside the autoclave chamber. Using RO water prevents equipment damage, ensures efficient steam generation, and improves the reliability of sterilization cycles. Under improper conditions, heat-resistant bacterial spores, particularly from *Bacillus* and *Clostridium* species, can survive and remain infectious (15). For this reason, hospitals and laboratories are encouraged to routinely verify the effectiveness of their sterilization processes. Regular testing not only ensures that equipment and biomedical waste are safely treated but also serves as an essential safeguard against infection outbreaks. In essence, continuous evaluation of sterilization performance—through proper monitoring, validated indicators, and high-quality water usage—is fundamental for protecting patients, healthcare workers, and the wider community.

MATERIALS AND METHODS

Institutional approval: Institutional approval was obtained from the research ethics committee of the hospital for conducting this research.

Study duration: This research was conducted from 6th August to 20th October, 2025, at the Laboratory Medicine Department.

Study population/area: Biomedical waste samples were collected from 5 different locations (where wastes are generated), including the operation theatre (OT), outpatient department (OPD), dressing unit, and diagnostic laboratory. In addition, samples of hospital drinking water and RO water were collected to assess microbial load. Environmental monitoring in the OT was performed by collecting from the OT floor, trays, and bed surface swabs. A total of 17 sterilization cycles were observed to assess sterilization efficacy with different types of quality indicators, such as biological indicator, chemical indicators, bowie dick test pack, and physical report. Also swab sample was collected from surgical instruments to assess microbial presence after sterilization.

Sample size calculation: Due to resource and time limitations, only one hospital was selected to conduct this research. In all the areas where biomedical wastes were generated, samples were collected. For environmental monitoring, a sample was taken from the operation theatre, considering the most critical area of the hospital. Due to time limitations, 17 sterilization cycles were observed to assess sterilization effectiveness, and one-time sampling of RO and drinking water.

Material used: For isolation of microorganisms, Nutrient Agar, Blood Agar, SS Agar, and Mac Conkey agar were used, and Muller Hinton agar was used for antimicrobial susceptibility test. All the media used were both FDA & CE certified, brand: Hi-media, Origin: India. All the antibiotics were CE certified, Brand: Oxoid, Origin: UK. For sterilization monitoring, all the sterilization quality assurance tools, such as chemical indicators, biological indicators, bowie dick test pack, etc., were CE certified, brand: Sterivision, Origin: Turkey. Equipment used for this study, such as incubator, biosafety cabinet, autoclave were also CE marked, Brand: Biobase, Origin: China.

Laboratory procedure: All the samples of biomedical waste swabs, environmental monitoring swabs, sterile surgical instrument swabs, and water, both drinking water as well as RO water for surgical instruments reprocessing, were inoculated onto Nutrient agar first and incubated for 24-48 hours at 37°C for primary culture (16). The growth samples were then sub-cultured to selective media such as MacConkey agar, SS Agar, and Blood agar and incubated for 24-48 hours at 37°C (17). Then, the positive pathogenic samples identified by physical monitoring as well as Gram staining were inoculated onto Muller-Hinton agar and incubated for 24 hours at 37°C for antibiotic susceptibility testing (18, 19). For sterilization efficacy monitoring, all the cycles were physically observed with the colour change of chemical indicators, bowie, dick test pack, and the physical report generated from the autoclave in real time (20, 21). Biological indicators were incubated at 56°C for 24-48 hours to observe the growth (22, 23).

Figure 1: Sample collection and inoculation.

Statistical analysis: IBM SPSS software version 22 was used for statistical analysis of the findings.

RESULTS

Table 01 shows microorganisms were successfully isolated from several areas of the hospital, including drinking water, operation theatre (OT) beds and trays, and biomedical waste from the Laboratory. In contrast, RO water and OT floor samples showed no visible

growth on nutrient agar, suggesting the absence of viable microorganisms during sampling. The identification of these isolates was confirmed through the observation of colony morphology and Gram staining results. *Staphylococcus* spp., isolated from OT beds and trays, showed growth on nutrient agar, hemolytic activity on blood agar, and appeared as Gram-positive cocci. *Escherichia coli*, isolated from hospital drinking water, produced pink lactose-fermenting colonies on MacConkey agar and appeared as Gram-negative short rods. *Klebsiella* spp., isolated from Biomedical Waste Laboratory sample- 2 and Laboratory sample- 3, formed mucoid colonies on MacConkey agar and appeared as Gram-negative rods under microscopy. And after autoclaving, no growth was found.

Table 02 presents the antibiotic susceptibility results of the isolated bacteria. The *E. coli* isolate showed complete sensitivity to all tested antibiotics, indicating the absence of multidrug resistance. The isolate from Laboratory Sample-2 exhibited resistance to Cefuroxime but remained sensitive to all other antibiotics. In contrast, the isolate from the Biomedical Waste Laboratory Sample-3 was sensitive to every antibiotic tested. Similarly, *Staphylococcus aureus* isolated from the OT tray and bed also showed full sensitivity to all tested antibiotics, reflecting an overall low level of antibiotic resistance among the studied samples.

Table 03 shows that a total of 17 sterilization cycles were observed, and all cycles achieved complete sterilization, demonstrating the reliability of the autoclaves and their adherence to international sterilization standards. All sterilization indicators—autoclave tape, physical reports, chemical indicators (such as indicator strips and Bowie-Dick test packs), and biological indicators—uniformly showed the expected results. Proper color changes were observed in the chemical indicators, and no microbial growth was detected in the biological indicator ampoules after 48 hours of incubation at 56°C, confirming that all sterilization cycles were 100% effective.

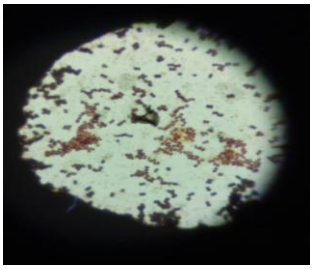

Figure 2: Observation of Gram-positive cocci under the microscope.

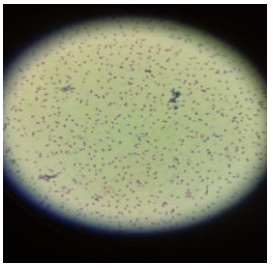

Figure 3: Observation of Gram-negative rod under the microscope.

Figure 4: Class N & Class B type sterilizer.

Table 1: Microbial growth and identification on nutrient, selective, and differential media supported by Gram staining.

Type of sample	Name of the specimen	Result (Nutrient Agar)	Result (Selective Media)	Confirmation Criteria (Gram Staining)
Environmental Monitoring	Operation Theatre (OT) Bed	A few	*Staphylococcus aureus*	Gram-positive cocci
	Operation Theatre (OT) Tray	A few	*Staphylococcus aureus*	Gram-positive cocci
	Operation Theatre (OT) Floor	No Growth	Not Done	Nil
Biomedical Wastes before Autoclave	Lab – 1	No Growth	Not Done	Nil
	Lab – 2	Profuse	*Klebsiella* spp.	Gram-negative rod
	Lab – 3	Moderate	*Klebsiella* spp.	Gram-negative rod
	OT	No Growth	Not Done	Nil
Biomedical Wastes after Autoclave	Lab – 1	No Growth	Not Done	Nil
	Lab – 2	No Growth	Not Done	Nil
	Lab – 3	No Growth	Not Done	Nil
	OT	No Growth	Not Done	Nil
Water	Drinking Water	Profuse	*E. coli* (Plenty)	Gram-negative rod
	RO Water	No Growth	Not Done	Nil

Table 2: Assessment of antibiotic susceptibility patterns.

Type of Sample	Name of Organism	Cefotaxime (CTX 30 µg)	Cefixime (CFM 30 µg)	Ceftriaxone (CRO 30 µg)	Levofloxacin (LEV 5 µg)	Cefuroxime (CXM 30 µg)	Ciprofloxacin (CIP 5 µg)
Drinking water	*Escherichia coli*	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Biomedical Wastes LAB-2	*Klebsiella* spp.	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Sensitive
Biomedical WastesLAB-3	*Klebsiella* spp.	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Environmental Monitoring OT Tray - 1	*Staphylococcus aureus*	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Environmental Monitoring OT Bed - 1	*Staphylococcus aureus*	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive

Table 3: Evaluation of autoclave sterilization effectiveness.

Cycle No	Type of sterilizer	Name of Medical Devices	Name of Packing Material	BD test Sheet	C.I Strip	Autoclave Tape	Sterilization Reel Indicator	Physical Report	Remark
1	Class N	Gown & Eye site	Surgical Rubber	Passed	Passed	Passed	Passed	Passed	Successful
2	Class B	Operation instruments & cotton	Surgical Rubber	Passed	Passed	Passed	Passed	Passed	Successful
3	Class N	Gown & Eye site	Surgical Rubber	Passed	Passed	Passed	Passed	Passed	Successful
4	Class B	Operation instruments & cotton	Surgical Rubber & Cotton Pad	Passed	Passed	Passed	Passed	Passed	Successful
5	Class N	Gown & Eye site	Surgical Rubber	Passed	Passed	Passed	Passed	Passed	Successful
6	Class B	Operation instruments & cotton	Surgical Rubber	Passed	Passed	Passed	Passed	Passed	Successful
7	Class N	Gown & Eye site	Surgical Rubber	Passed	Passed	Passed	Passed	Passed	Successful
8	Class B	Operation instruments & cotton	Surgical Rubber & Cotton Pad	Passed	Passed	Passed	Passed	Passed	Successful
9	Class B	Operation instruments & cotton	Surgical Rubber	Passed	Passed	Passed	Passed	Passed	Successful
10	Class N	Gown & Eye site	Surgical Rubber	Passed	Passed	Passed	Passed	Passed	Successful
11	Class B	Operation instruments & cotton	Surgical Rubber & Cotton Pad	Passed	Passed	Passed	Passed	Passed	Successful
12	Class N	Gown & Eye site	Surgical Rubber	Passed	Passed	Passed	Passed	Passed	Successful
13	Class B	Operation instruments & cotton	Surgical Rubber & Cotton Pad	Passed	Passed	Passed	Passed	Passed	Successful
14	Class B	Operation instruments & cotton	Surgical Rubber & Cotton Pad	Passed	Passed	Passed	Passed	Passed	Successful
15	Class N	Gown & Eye site	Surgical Rubber	Passed	Passed	Passed	Passed	Passed	Successful
16	Class N	Gown & Eye site	Surgical Rubber	Passed	Passed	Passed	Passed	Passed	Successful
17	Class B	Operation instruments & cotton	Surgical Rubber & Cotton Pad	Passed	Passed	Passed	Passed	Passed	Successful

DISCUSSION & CONCLUSIONS

Standard management of biomedical waste, proper sterilization practices, and regular environmental and water quality monitoring are essential components of infection control practices within healthcare facilities. These measures not only protect patients and healthcare workers but also help prevent the spread of infectious agents in the hospital environment (24). This study was conducted to assess the microbiological characteristics of biomedical waste produced in a specialized eye hospital in Dhaka, alongside the evaluation of the water quality of drinking water, the environmental cleanliness of the operating theatre, and the sterilization efficiency of surgical instruments. Additionally, the antibiotic profile of the isolated microorganisms was examined. The overall findings contribute valuable insights into improving infection prevention and maintaining a safe healthcare environment (24-26).

In this study, *Escherichia coli* was presumptively identified in the hospital's drinking water sample. The organism demonstrated typical colony characteristics (pink, sticky in MacConkey agar) on selective media and was microscopically confirmed as a Gram-negative short rod. This finding is consistent with the observations of Nowicki *et al.* (2021) and Wispriyono *et al.* (2021), who recognized *E. coli* as a dependable indicator of fecal pollution in drinking water sources (27, 28). The detection of *E. coli* indicates that the hospital water supply may have been subjected to a certain degree of contamination, potentially arising from environmental exposure or improper handling practices. Antibiotic susceptibility testing revealed that the *E. coli* isolate was sensitive to all antibiotics tested, signifying the absence of multidrug resistance (MDR). This outcome aligns with the findings of Zarić *et al*. (2023), who reported antibiotic-sensitive *E. coli* strains in hospital and community water samples (29). However, it contrasts with the results of Seguni *et al*. (2023), who detected antibiotic-resistant *E. coli* in hospital wastewater, suggesting that resistance profiles may vary depending on environmental origin and exposure to antimicrobial agents (30). Additionally, *Klebsiella* species were recovered from the Biomedical Waste Laboratory sample 2 and Laboratory sample 3. These isolates exhibited the typical morphological and biochemical traits of *Klebsiella*, supporting the report by Meral *et al*. (2025), who demonstrated the ability of *Klebsiella pneumoniae* to persist in humid hospital settings, including biomedical waste (31). Antibiotic sensitivity analysis showed that the isolate from Laboratory sample 2 exhibited resistance to cefuroxime but remained sensitive to other antibiotics, while the isolate from Biomedical Waste Laboratory sample 3 was sensitive to all tested antibiotics. The observed partial resistance to cefuroxime corresponds with the results of Karungamye *et al*. (2023), who described similar cephalosporin resistance patterns in *Klebsiella* isolates (32). The variation in antibiotic resistance between the two isolates may be attributed to differences in waste management, storage conditions, and moisture content, which could affect bacterial survival and exposure to residual antimicrobial substances. The findings show positive correlation with Meral *et al*. (2025) and Karungamye *et al*. (2023), emphasizing *Klebsiella* adaptability in biomedical waste environments (31, 32), but are in contrast with Singh *et*

al. (2020), who reported a lower incidence of *Klebsiella* in healthcare facilities maintaining efficient sterilization and disinfection protocols (33). The detection of partial resistance among *Klebsiella* isolates serves as an early indication of potential emergence of broader resistance.

Therefore, continuous microbiological surveillance of hospital water and biomedical waste is essential to mitigate environmental contamination and control the spread of antibiotic-resistant microorganisms. *Staphylococcus* spp. was isolated from the operation theatre (OT) beds and trays and was identified as Gram-positive cocci. All isolates were found to be sensitive to the antibiotics tested, suggesting the absence of multidrug resistance and reflecting the effectiveness of infection control and sterilization measures within the hospital environment (34). This observation is consistent with the findings of Otter *et al*. (2022) and Reddy *et al*. (2023), who reported that *Staphylococcus* spp. is among the most common environmental contaminants capable of persisting on dry surfaces for extended periods (35, 36). Similarly, Gupta *et al*. (2021) emphasized that the frequency and efficiency of cleaning and disinfection directly affect surface contamination levels, highlighting the necessity of maintaining rigorous hygiene standards in hospital settings (37). The present study's results also align with Ibrahim *et al*. (2020), who observed that healthcare facilities implementing regular environmental cleaning and microbiological surveillance exhibited reduced bacterial loads and antibiotic-sensitive isolates (38). In contrast, Singh *et al*. (2019) and Bora *et al*. (2021) documented the occurrence of antibiotic-resistant *Staphylococcus aureus* strains in hospitals with inadequate sanitation and irregular monitoring, underscoring the link between insufficient infection control and resistance emergence (39, 40). The detection of antibiotic-sensitive *Staphylococcus* spp. and the absence of resistant strains in this study indicate that the hospital's current cleaning, disinfection, and sterilization practices are functioning effectively. Nevertheless, continuous microbiological surveillance, combined with routine staff training and periodic environmental assessments, remains essential to sustain a contamination-free OT environment and ensure patient safety (35, 37).

Figure 5: Result of sterilization effectiveness in physical, chemical, and biological indicators.

Sterilization effectiveness is a fundamental component of hospital infection control programs. It plays a vital role in preventing healthcare-associated infections (HAIs) and maintaining patient safety by ensuring that all reusable medical instruments and equipment are completely free from viable microorganisms (41). In the present study, all 17 sterilization cycles demonstrated complete success, confirming the reliability of the autoclaves and their compliance with internationally accepted sterilization standards (42). Effective sterilization not only minimizes the risk of pathogen transmission but also enhances the overall quality and safety of healthcare services (43). Similar conclusions were reported in the World Health Organization (WHO) guidelines and by Rutala *et al*. (2019), who emphasized that strict adherence to sterilization protocols is essential for effective infection prevention and control in hospital environments (41, 42).

When compared with earlier research, the present findings show a marked difference. Panta *et al*. (2019) reported that 71% of sterilization cycles were ineffective when assessed using biological indicators, and 69.8% failed based on Class 5 chemical indicators in Nepalese public hospitals. This variation may be explained by the well-regulated operational conditions, regular autoclave maintenance, and consistent monitoring practices employed in the current study. Conversely, the study by Panta *et al*. (2019) found that variations in autoclave models, inadequate pressure generation, and limited staff training were key contributors to ineffective sterilization outcomes (43). Similarly, Sifat Uz Zaman *et al*. (2021) observed a 2% biological indicator failure rate in steam sterilization at a cardiac hospital in Bangladesh, attributing it to insufficient monitoring procedures and a lack of trained personnel (44). Moreover, Zhang *et al*. (2022) and Lee *et al*. (2023) highlighted that reliable sterilization outcomes largely depend on regular equipment calibration, the use of multiple indicator systems, and comprehensive staff training initiatives. In contrast to these findings, the 100% sterilization success rate achieved in the present research demonstrates strict procedural adherence, multi-indicator validation, and systematic equipment maintenance, all contributing to consistently superior sterilization performance (45, 46).

Special focus should be given to biomedical waste management and drinking water. Due to time and resource limitations, this cross-sectional research could not be explored with a larger sample size. In future, number of government and private hospitals with larger sample size will be enrolled to conduct this study.

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