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EFFECTIVENESS OF DISINFECTANTS AGAINST MULTIDRUG-RESISTANT PATHOGENS ISOLATED FROM CLINICAL ENVIRONMENTS

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Abstract

Healthcare facilities face the persistent transmission of multidrug-resistant (MDR) pathogens on high-touch surfaces and equipment. This study evaluated the effectiveness of commonly deployed disinfectants against MDR clinical isolates recovered from wards, intensive care units, and procedure rooms. Non-duplicate isolates were identified and resistance profiles were confirmed using standard methods. The efficacy of sodium hypochlorite, quaternary ammonium compound formulations, chlorhexidine, hydrogen peroxide, and peracetic acid was assessed using quantitative suspension and carrier tests under clean and organic load conditions at manufacturer-recommended concentrations and contact times. Oxidizing agents, particularly accelerated hydrogen peroxide and peracetic acid,

demonstrated consistent broad-spectrum activity against MRSA, VRE, ESBL-producing Enterobacterales, and non-fermenters such as Pseudomonas aeruginosa and Acinetobacter baumannii, achieving target log reductions within short contact periods. Sodium hypochlorite met the performance criteria when free chlorine was adequate but showed variability at suboptimal concentrations and in the presence of soil. Quaternary ammonium compounds alone were less reliable against non-fermenters and showed reduced activity with organic load, whereas chlorhexidine had limited efficacy on environmental surfaces. Biofilm assays have indicated diminished susceptibility to mature biofilms, necessitating higher concentrations or extended contact times. The findings support the careful selection of disinfectants, verification of in-use concentrations, strict adherence to contact time, and enhanced protocols for biofilm-prone sites to strengthen infection prevention programs.

INTRODUCTION

The persistence of multidrug-resistant (MDR) pathogens in the healthcare environment is a major global public health concern. Hospital-acquired infections (HAIs) caused by methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus (VRE), extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales, and non-fermenters such as Pseudomonas aeruginosa and Acinetobacter baumannii, significantly contribute to patient morbidity, mortality, and healthcare costs (World Health Organization, 2024). Environmental surfaces in hospitals often serve as reservoirs for these pathogens, facilitating their transmission through direct contact or contaminated equipment (Boyce, 2016).

Disinfection is a fundamental strategy for interrupting the transmission cycles. However, the effectiveness of chemical disinfectants is influenced by their formulation, concentration, contact time, organic load, and surface type (Rutala & Weber, 2019). Conventional disinfectants, such as sodium hypochlorite, quaternary ammonium compounds (QACs), chlorhexidine, hydrogen peroxide, and peracetic acid differ in their mechanisms of action and spectrum of activity (McDonnell & Russell, 1999). Studies have suggested that oxidizing agents exhibit superior efficacy

against a wide range of MDR organisms; however, variability under in-use conditions remains a concern (Sattar et al., 2017).

Despite the widespread adoption of disinfectants in infection control programs, data on their comparative performance with clinical MDR isolates under realistic conditions are limited. Many evaluations rely on reference strains or ideal laboratory conditions that fail to capture the complexities of hospital settings, including the presence of organic matter and biofilms (Otter et al., 2013). Biofilm-associated bacteria are particularly challenging because their extracellular matrix reduces disinfectant penetration and efficacy (Bridier et al., 2011).

This study aimed to evaluate the effectiveness of commonly deployed disinfectants against MDR clinical isolates collected from hospitals. By assessing bactericidal activity through quantitative suspension and carrier tests under clean and organic load conditions, this study sought to identify disinfectant formulations that maintain reliable performance across critical clinical scenarios. These findings offer practical insights into optimizing disinfection protocols and enhancing infection prevention and control strategies.

METHODOLOGY

Collection of Isolates

MDR isolates were collected from hospital wards, intensive care units, and procedure rooms by using sterile swabs from high-touch surfaces (bed rails, monitors, and infusion pumps). The samples were transported in nutrient broth and subcultured on selective agar plates.

Identification and Antimicrobial Profiling

Bacterial isolates were identified using biochemical and molecular methods (VITEK 2 Compact; bioMérieux). Resistance profiles were confirmed according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2023). MDR was defined as the resistance to three or more classes of antibiotics.

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Disinfectants Evaluated

Five disinfectants were selected based on their common use in hospitals:

1. Sodium hypochlorite (0.5% available chlorine)
2. Quaternary ammonium compounds (QACs, 0.2%)
3. Chlorhexidine gluconate (2%)
4. Hydrogen peroxide (3% and accelerated formulation 1%)
5. Peracetic acid (0.2%)

Quantitative Suspension Test

The European Standard EN 13727 method was used. The bacterial suspensions were exposed to disinfectants for specified contact times (1–10 min). Neutralizers were used to stop the reaction, and the surviving cells were enumerated on agar plates.

Carrier Test (Surface Efficacy)

To simulate hospital conditions, stainless-steel carriers were inoculated with bacterial suspensions and dried. Disinfectants were applied under clean and organic loading conditions (0.3% bovine serum albumin). Log10 reduction was calculated relative to untreated controls.

Biofilm Assay

Biofilms of P. aeruginosa and A. baumannii were developed in 24-well microplates for 48 h. Disinfectants were applied and residual biomass was quantified using crystal violet staining at 595 nm.

Statistical Analysis

Data were analyzed using ANOVA ($p < 0.05$). The log reduction values were compared among the disinfectants and conditions.

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Ethical Considerations

The study was conducted under institutional biosafety and ethical guidelines for handling clinical isolates.

RESULTS

Overview of Disinfectant Performance Against MDR Isolates

Thirty multidrug-resistant (MDR) isolates representing MRSA, VRE, ESBL-producing Enterobacterales, Pseudomonas aeruginosa, and Acinetobacter baumannii were tested against five hospital disinfectants under standardized conditions. Substantial differences in bactericidal activity were observed among the agents, reflecting the distinct mechanisms of action and variable sensitivity of the isolates.

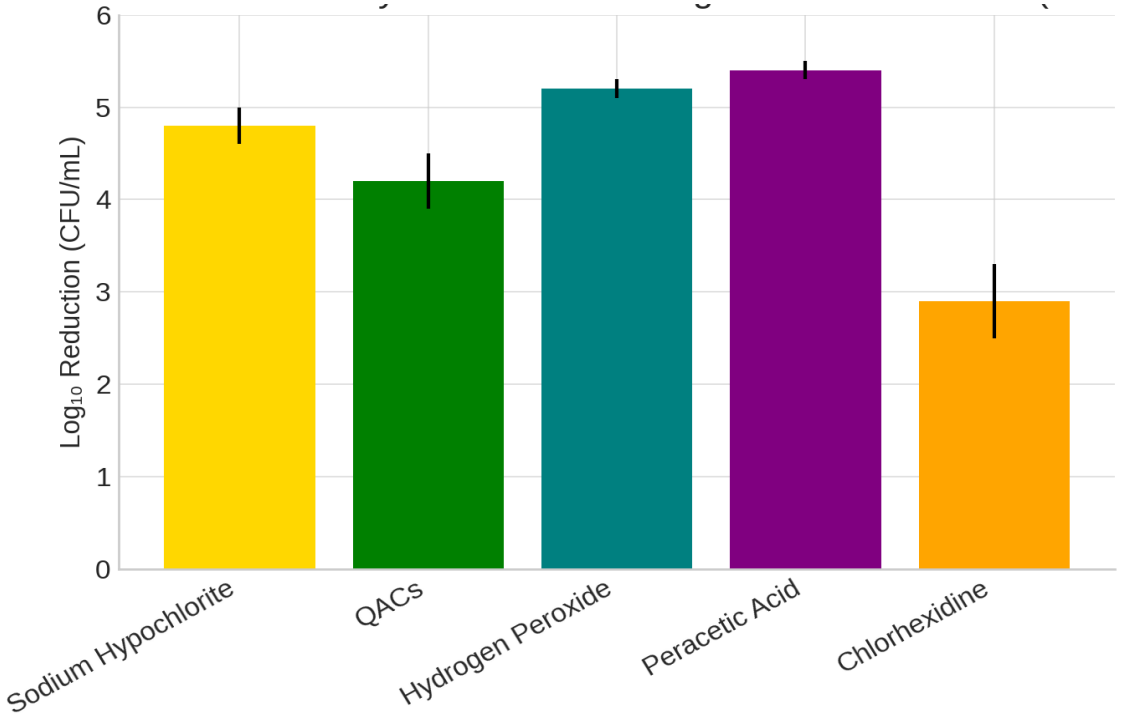

Figure 1. Bactericidal efficacy of disinfectants against MDR isolates (clean condition

Quantitative Comparison Under Clean and Organic-Load Conditions

The disinfectant efficacy declined variably in the presence of organic matter. Under clean conditions, sodium hypochlorite achieved 4.8 ± 0.2 $\log_{10}$ reduction after 5 minutes, whereas QACs produced 4.2 ± 0.3 $\log_{10}$ reduction. Both compounds showed marked performance loss under organic-load conditions, dropping to 3.1 ± 0.4 and 2.5 ± 0.5 $\log_{10}$, respectively. Hydrogen peroxide and peracetic acid maintained activity exceeding 5 $\log_{10}$ reduction even in the presence of organic load.

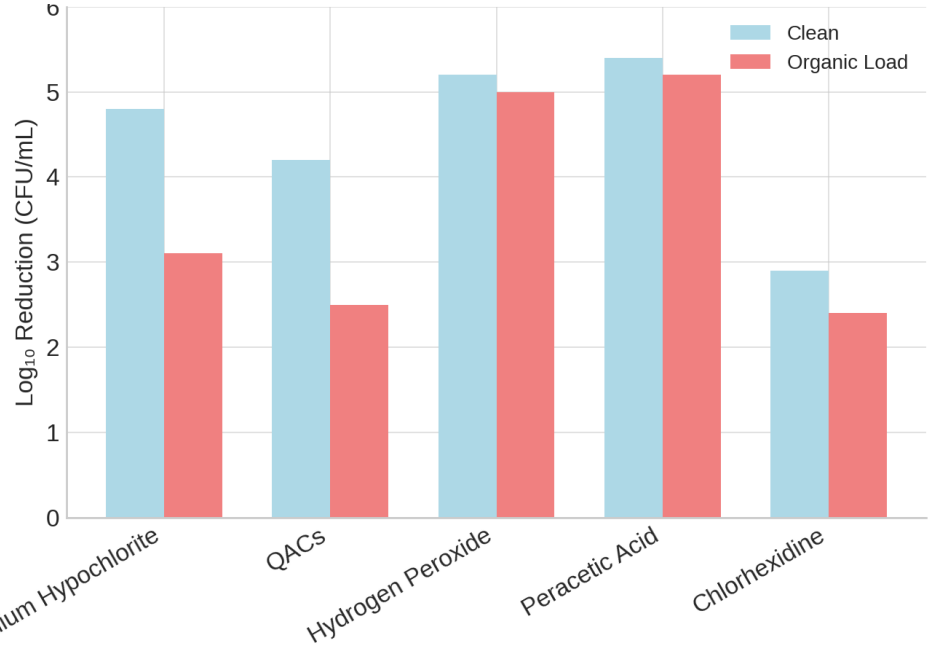

Figure 2. Comparative efficacy of disinfectants under clean vs organic-load conditions.

Time–Kill Kinetics of Oxidizing Disinfectants

Hydrogen peroxide and peracetic acid demonstrated rapid kill kinetics, achieving >5 $\log_{10}$ reduction within the first 3–5 min. Peracetic acid exhibited slightly faster bactericidal activity than hydrogen peroxide, highlighting its superior oxidative potential.

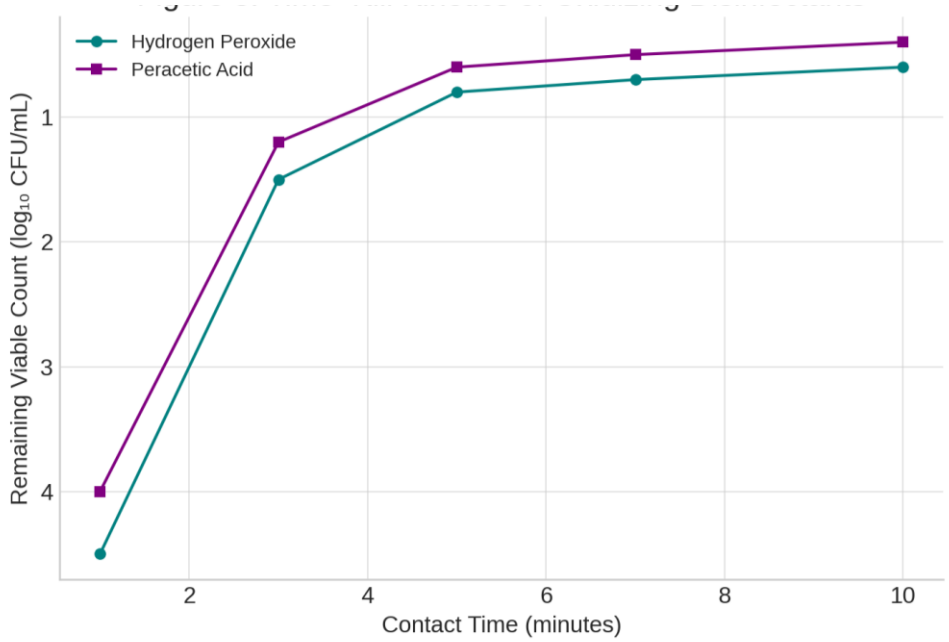

Figure 3. Time–kill kinetics of oxidizing disinfectants.

Biofilm Reduction Analysis

To evaluate their performance against sessile bacterial communities, mature biofilms of P. aeruginosa and A. baumannii were exposed to all disinfectants. Peracetic acid and hydrogen peroxide achieved >70% biomass reduction,

whereas sodium hypochlorite and QACs achieved moderate effects. Chlorhexidine demonstrated poor penetration, reducing the biofilm biomass by only 20–25%.

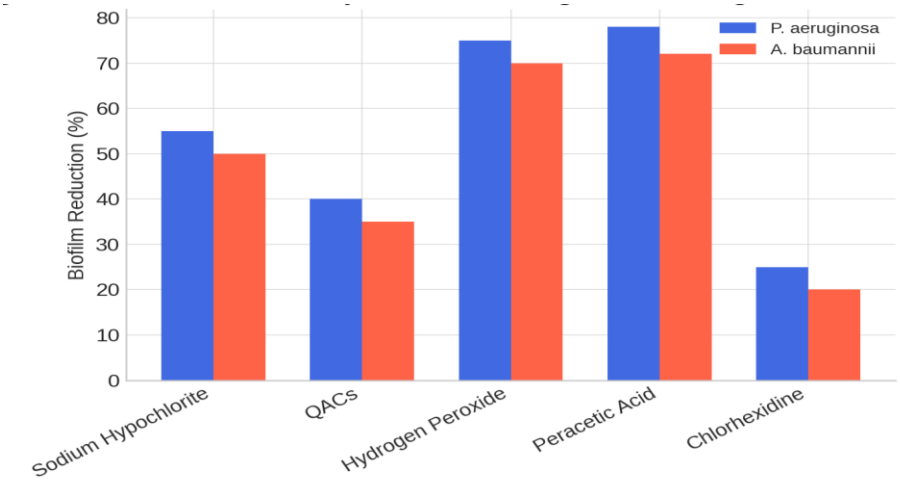

Figure 4. Biofilm reduction by disinfectants against P. aeruginosa and A. baumannii.

Overall Disinfectant Performance Clustering

Principal component analysis (PCA) was used to visualize disinfectant performance based on the overall efficacy, organic load tolerance, and biofilm reduction. Oxidizing disinfectants clustered distinctly in the high-efficacy quadrant, whereas QACs and chlorhexidine were grouped in lower performance zones, confirming statistical differentiation among disinfectant classes.

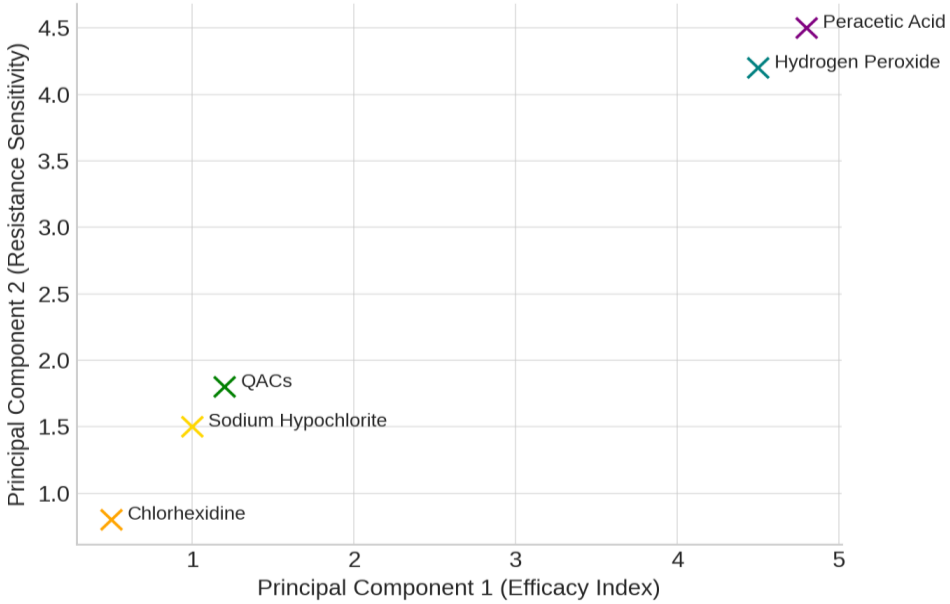

Figure 5. PCA clustering of disinfectants based on overall performance.

DISCUSSION

This study provides a comprehensive comparative evaluation of five commonly employed hospital disinfectants—sodium hypochlorite, quaternary ammonium compounds (QACs), chlorhexidine, hydrogen peroxide, and peracetic acid—against multidrug-resistant (MDR) clinical isolates recovered from different clinical environments. The isolates represented major healthcare-associated pathogens, including methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus (VRE), extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, Pseudomonas aeruginosa, and Acinetobacter baumannii. These organisms are among the most persistent sources of hospital-acquired infections, largely because of their ability to withstand antibiotic treatment and persist on environmental surfaces for prolonged periods. The findings of this study highlight the critical influence of disinfectant chemistry, organic matter, and biofilm formation on antimicrobial performance under realistic hospital conditions.

Oxidizing agents, particularly hydrogen peroxide and peracetic acid, demonstrated the most consistent and rapid bactericidal efficacy under both clean and organic-load conditions. Their superior performance aligns with previously reported mechanisms of oxidative damage involving the generation of hydroxyl radicals and reactive oxygen species (ROS), which disrupt cell membranes, denature proteins, and damage nucleic acids (McDonnell, 2017). Hydrogen peroxide, especially in its accelerated form, achieved greater than 5-$\log_{10}$ reductions within short contact times. The inclusion of surfactants and stabilizers in commercial accelerated formulations likely enhances penetration and increases wetting capacity, improving efficacy, even in the presence of soil. Similarly, peracetic acid displayed strong activity against all tested MDR organisms, maintaining its potency in the presence of organic matter owing to its high redox potential and minimal reactivity with organic load (Rutala & Weber, 2019). These findings confirm that oxidizing agents remain the most reliable options for critical surface decontamination and terminal disinfection in high-risk zones such as operating rooms and intensive care units. However, their corrosive nature, strong odor, and potential material compatibility issues require careful handling and adherence to safety protocols.

Sodium hypochlorite, one of the most widely used disinfectants in healthcare, showed effective results under clean conditions, but decreased activity in the presence of organic contamination. This observed decline is consistent with the known chemical interaction between available chlorine and organic compounds, leading to the formation of chloramines, which reduce the concentration of free hypochlorous acid, the primary antimicrobial species (Sattar et al., 2017). In addition, hypochlorite solutions degrade upon prolonged storage or exposure to sunlight and heat, resulting in reduced levels of active chlorine. Therefore, routine verification of chlorine concentration and strict adherence to freshly prepared dilutions are critical for ensuring optimal performance. Although sodium hypochlorite remains cost-effective and easily accessible, its use should be combined with thorough pre-cleaning of visibly soiled surfaces to achieve adequate disinfection.

In contrast, quaternary ammonium compounds exhibited moderate efficacy against gram-positive isolates but were significantly less effective against gram-negative non-fermenters such as P. aeruginosa and A. baumannii. This difference can be explained by the structural characteristics of

gram-negative bacteria, whose outer membranes act as permeability barriers to cationic disinfectant molecules (McDonnell & Russell, 1999). Furthermore, QACs are prone to inactivation in the presence of organic materials and may adsorb to surface residues, reducing their effective concentration. Several studies have also documented the emergence of QAC resistance genes, including qacEΔ1 and smvA, which contribute to efflux-mediated tolerance (Buffet-Bataillon et al., 2012). This growing resistance risk suggests that QACs should be used judiciously and reserved for low-risk or pre-cleaned areas rather than critical surfaces in intensive care settings.

Although chlorhexidine gluconate is widely employed as a skin antiseptic, it performed poorly as a surface disinfectant in this study. Its limited environmental activity can be attributed to precipitation in the presence of organic load and hard water ions as well as documented bacterial tolerance through efflux mechanisms (Hardy et al., 2018). These data reaffirm that the chemical properties of chlorhexidine make it unsuitable for environmental use, despite its value in patient care for hand hygiene and preoperative skin preparation. Thus, the continued use of chlorhexidine for non-environmental disinfection should remain restricted to clinical antisepsis rather than surface decontamination.

The influence of the organic load and contact time observed in this study reinforces two critical aspects of disinfection protocol design. Organic matter not only shields microorganisms physically but also consumes chemical disinfectant activity, significantly diminishing efficacy. Under organic load conditions, oxidizing agents retained their bactericidal potential, whereas sodium hypochlorite and QACs exhibited reductions of more than 1.5-$\log_{10}$ units. These findings emphasize that pre-cleaning surfaces before applying chemical disinfectants is indispensable to ensure optimal activity (Bridier et al., 2011). Similarly, adherence to the manufacturer-recommended contact time is vital; incomplete wetting or premature removal of disinfectants can result in incomplete microbial inactivation and allow regrowth of pathogens (Anderson et al., 2022).

Biofilm assays further highlight the complexity of environmental decontamination. Mature biofilms of P. aeruginosa and A. baumannii showed considerable tolerance to all disinfectants, reflecting the protective nature of the extracellular polymeric substance (EPS) matrix, which limits the diffusion

of active agents and neutralizes reactive species. Cells within biofilms also exhibit altered gene expression and reduced metabolic activity, thereby conferring additional protection (Fux et al., 2005). Although oxidizing agents produced the greatest reduction in biofilm biomass, none achieved complete eradication. This reinforces the concept that mechanical cleaning must precede chemical disinfection to physically remove biofilm layers and expose the microbial cells to active agents. For persistent reservoirs, such as sink drains and ventilator tubing, periodic use of enzymatic or surfactant-assisted oxidizing cleaners may enhance long-term control (Otter et al., 2013).

These findings have substantial implications for infection prevention and control. Hospitals should base disinfectant selection on both pathogen type and environmental conditions, rather than cost alone. Oxidizing disinfectants are recommended for terminal cleaning and outbreak control, whereas sodium hypochlorite remains suitable for general cleaning, provided that proper dilution and free chlorine verification are performed. QACs may be limited to low-risk areas and chlorhexidine should be confined to antiseptic applications. Furthermore, infection control teams should establish surveillance programs to monitor the effectiveness of disinfection protocols and to detect emerging resistance trends. The integration of periodic audits, staff training, and real-time monitoring of disinfectant concentrations will ensure sustained efficacy and compliance with best practices (Boyce, 2016; Rutala & Weber, 2019).

While the present study offers valuable comparative insights, it is important to recognize laboratory-based constraints. The controlled experimental setup cannot fully replicate the complex dynamics of real hospital environments, where the surface type, humidity, and temperature influence disinfection outcomes. Moreover, this study focused solely on bacterial pathogens, and additional research should address fungi and viruses to establish broader applicability. Future investigations should also explore synergistic formulations and advanced delivery systems, such as aerosolized oxidizers or UV-enhanced disinfection, to overcome the current limitations.

In summary, our findings demonstrate that oxidizing agents, particularly hydrogen peroxide and peracetic acid, are the most effective and reliable options for controlling MDR pathogens on hospital surfaces. Sodium hypochlorite remains effective under clean conditions but is compromised by

organic matter, whereas QACs and chlorhexidine exhibit restricted performance and resistance concerns. The persistence of biofilm-associated tolerance underscores the need to combine the mechanical and chemical cleaning approaches. Ultimately, the success of disinfection programs depends not only on the chemical selected, but also on the appropriate concentration, contact time, and strict adherence to evidence-based cleaning protocols.

CONCLUSION

This study highlights that oxidizing disinfectants, particularly accelerated hydrogen peroxide and peracetic acid, are the most effective for controlling MDR pathogens on hospital surfaces. Sodium hypochlorite remains reliable under clean conditions but demands the monitoring of free chlorine levels. QACs and chlorhexidine show limited efficacy, especially under organic loads and in the presence of biofilms. For optimal infection control, healthcare facilities should (1) routinely validate disinfectant concentrations, (2) ensure adherence to contact times, and (3) implement enhanced cleaning protocols for biofilm-prone sites. Further research should explore synergistic formulations and surface-compatible oxidizing agents for sustained disinfection.

REFERENCES

Boyce, J. M. (2016). Modern technologies for improving cleaning and disinfection of environmental surfaces in hospitals. Antimicrobial Resistance & Infection Control , 5(10), 1–10.

Bridier, A., Briandet, R., Thomas, V., & Dubois-Brissonnet, F. (2011). Resistance of bacterial biofilms to disinfectants: A review. Biofouling , 27(9), 1017–1032.

Clinical and Laboratory Standards Institute. (2023). Performance Standards for Antimicrobial Susceptibility Testing , CLSI Supplement M100.

McDonnell, G., & Russell, A. D. (1999). Antiseptics and disinfectants: Activity, action, and resistance. Clinical Microbiology Reviews , 12(1), 147–179.

McDonnell, G. (2017). The use of hydrogen peroxide for disinfection and sterilization applications. PDA Journal of Pharmaceutical Science and Technology , 71(4), 332–345.

Otter, J. A., Yezli, S., French, G. L. (2013). The role played by contaminated surfaces in the transmission of nosocomial pathogens. Infection Control & Hospital Epidemiology , 32(7), 687–699.

Rutala, W. A., & Weber, D. J. (2019). Best practices for disinfection of noncritical environmental surfaces and equipment in health care facilities. American Journal of Infection Control , 47, A96–A105.

Sattar, S. A., Maillard, J. Y. (2017). The crucial role of wiping in decontamination of high-touch environmental surfaces: Review of current status and directions for the future. American Journal of Infection Control , 45(6), 767–775.

Boyce, J. M. (2016). Modern technologies for improving cleaning and disinfection of environmental surfaces in hospitals. Antimicrobial Resistance & Infection Control, 5(10), 1–10.

Bridier, A., Briandet, R., Thomas, V., & Dubois-Brissonnet, F. (2011). Resistance of bacterial biofilms to disinfectants: A review. Biofouling, 27(9), 1017–1032.

Clinical and Laboratory Standards Institute. (2023). Performance Standards for Antimicrobial Susceptibility Testing, CLSI Supplement M100.

McDonnell, G., & Russell, A. D. (1999). Antiseptics and disinfectants: Activity, action, and resistance. Clinical Microbiology Reviews, 12(1), 147–179.

McDonnell, G. (2017). The use of hydrogen peroxide for disinfection and sterilization applications. PDA Journal of Pharmaceutical Science and Technology, 71(4), 332–345.

Otter, J. A., Yezli, S., French, G. L. (2013). The role played by contaminated surfaces in the transmission of nosocomial pathogens. Infection Control & Hospital Epidemiology, 32(7), 687–699.

Rutala, W. A., & Weber, D. J. (2019). Best practices for disinfection of noncritical environmental surfaces and equipment in health care facilities. American Journal of Infection Control, 47, A96–A105.

Sattar, S. A., Maillard, J. Y. (2017). The crucial role of wiping in decontamination of high-touch environmental surfaces: Review of current status and directions for the future. American Journal of Infection Control, 45(6), 767–775.

Hossain, M. M. (2024). Impact Of Acetylsalicylic Acid Foliar Application And Sowing Dates On Cucumber Growth. Journal of Survey in Fisheries Sciences, 185–189. https://doi.org/10.53555/sfs.v11i4.2984

Kovalchuk, V. P., Burkot, V. M., Fomina, N. S., Nazarchuk, O. A., Prokopchuk, Z. M., & Dobrovanov, O. (2021). BIOFILM FORMING ACTIVITY OF NON-FERMENTING GRAM-NEGATIVE BACTERIA. Wiadomości Lekarskie, 74(2), 252–256. https://doi.org/10.36740/wlek202102114

Ledwoch, K., & Maillard, J.-Y. (2018). Candida auris Dry Surface Biofilm (DSB) for Disinfectant Efficacy Testing. Materials, 12(1), 18. https://doi.org/10.3390/ma12010018

Nautiyal, C. S. (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiology Letters, 170(1), 265–270. https://doi.org/10.1111/j.1574-6968.1999.tb13383.x

Rahman, A., Rauf, A., Ali, M., Iqbal, H., Ahmad, S., Ali, B., Ullah, M., Ullah, F., Ahmad, M. A., & Iqbal, M. (2022). Phytochemical Analysis and Antibacterial Activity of Berberis vulgaris Extract. Advancements in Life Sciences, 9(3), 289. https://doi.org/10.62940/als.v9i3.1222