Comparative Study of Pathogen Removal and Environmental Safety in Public Toilet Sanitation (Bio fermented vs. Conventional Disinfectants)

Keerthanaa Vijayanand

Saigarden, Annanagar West extension, Chennai-101

DOI: https://doi.org/10.51244/IJRSI.2025.12110163

Received: 03 December 2025; Accepted: 10 December 2025; Published: 22 December 2025

ABSTRACT

This comparative case study examines the efficacy and environmental safety of biofermented (probiotic-based) cleaners versus conventional chemical disinfectants in the sanitation of public toilets—a critical facet of urban public health infrastructure. The analysis synthesizes evidence from over 40 peer-reviewed studies, focusing on pathogen removal rates, antimicrobial resistance (AMR) implications, operational sustainability, and life cycle environmental impacts. Findings show that biofermented cleaners achieve comparable or superior pathogen reduction and provide sustained microbial suppression through competitive exclusion, virtually eliminating the risk of AMR amplification—a major limitation of chemical disinfectants. Cost-benefit analysis using INR values highlights a potential 75% reduction in total operational costs when adopting biofermented systems at scale. Additionally, environmental impact assessments demonstrate that enzyme-based cleaners are fully biodegradable and produce negligible toxic residues, contrasting sharply with the high environmental burden and disinfection byproduct formation of chlorine- and quaternary ammonium-based disinfectants. The study concludes by recommending pilot implementation and regulatory recognition of biofermented cleaners for public sanitation in India, citing benefits for public health, environmental sustainability, and economic efficiency.

INTRODUCTION AND PROBLEM CONTEXT

Public toilets represent critical infrastructure for public health, yet they remain persistent reservoirs of pathogenic microorganisms that pose significant transmission risks to vulnerable populations.[1][2] This comprehensive case study comparative analysis examines the efficacy of biofermented (probiotic-based) cleaning systems versus conventional chemical disinfectants in reducing pathogen contamination and their respective environmental safety profiles in public toilet sanitation.

Biofermented cleaners (using Bacillus-based probiotics) demonstrate pathogen reduction rates comparable to or superior to conventional disinfectants, with an additional critical advantage: sustained pathogen suppression without antimicrobial resistance (AMR) generation.[1][3][4]

Conventional chemical disinfectants (sodium hypochlorite, quaternary ammonium compounds, phenolic compounds) show short-term efficacy but fail to prevent pathogen recontamination and promote the emergence of multidrug-resistant (MDR) pathogens. [2][5][6] Environmental impact assessment reveals that chemical disinfectants generate significant toxicity through their production, application, and residual effects, particularly affecting aquatic ecosystems. [7][8][9]

Biofermented systems offer a paradigm shift in sanitation approach: rather than annihilation-based disinfection, they employ competitive exclusion of pathogens through beneficial microbiome establishment.[4][10]

Public Toilets as Disease Transmission Vectors

Public toilets serve as high-traffic contact hubs where multiple pathogenic microorganisms accumulate on surfaces, creating substantial cross-infection risks.[1] Common pathogenic isolates recovered from public toilet environments include:[11][12][13]

• Gram-negative bacteria: Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacteriaceae

• Gram-positive bacteria: Staphylococcus aureus, Bacillus spp.

• Enteric pathogens: Salmonella spp., Proteus spp.

• Viral pathogens: SARS-CoV-2, norovirus, influenza viruses

• Fungal agents: Mold species in biofilms

The risks are particularly acute in urban areas with high foot traffic and in settings serving vulnerable populations (homeless shelters, transit stations, slums).[14][15] Public toilets with inadequate ventilation show bacterial contamination levels five times higher than ventilated facilities.[16]

Current Sanitation Challenges

Conventional Disinfectant Limitations:

1. Short-lived efficacy: Chemical disinfectants provide immediate pathogen reduction but fail to prevent rapid recontamination within 24-48 hours.[6][17]

2. Biofilm persistence: Pathogenic biofilms in toilet bowls and pipework resist chemical penetration, harboring viable pathogens beneath disinfectant action.[6][18]

3. Antimicrobial resistance (AMR): Widespread use of chemical disinfectants (particularly during and post-COVID-19) selects for resistant microorganisms, creating a public health crisis.[3][5][19]

4. Environmental toxicity: Chemical residues persist in wastewater, affecting aquatic ecosystems and human health through exposure pathways.[9][20][21]

Research Gap:

Limited comparative data exist on the long-term effectiveness and environmental impact of alternative sanitation approaches (biofermented cleaners) specifically in public toilet settings, particularly in developing country contexts.[1][22]

LITERATURE REVIEW

Comparative Efficacy

Probiotic-Based Sanitation (PBS) Systems

Mechanism of Action:

Probiotic-based cleaners utilize selected apathogenic Bacillus species and other beneficial microorganisms that:[1][3][4]

1. Outcompete pathogens through rapid colonization of surfaces, employing competitive exclusion mechanisms.[4][10]

2. Produce antimicrobial compounds (bacteriocins, organic acids) that suppress pathogenic proliferation.[3][4]

3. Enhance microbial diversity, establishing a stable, resilient microbiome resistant to pathogenic invasion.[4][18]

4. Degrade biofilms enzymatically, removing niches where pathogens shelter.[10][18]

Efficacy Data:

Caselli (2017) [4] reported that probiotic-based sanitation systems reduce surface pathogens 90% more than conventional disinfectants without promoting resistant species emergence. Ramos & Frantz (2023)[1] found that Bacillus-based PBS significantly reduces pathogen burden and antimicrobial-resistant genes in hospital environments, whereas conventional disinfectants show limited long-term efficacy.

D'Accolti et al. (2021)[23] demonstrated that Probiotic Cleaning Hygiene System (PCHS) inactivates 99.99% of enveloped viruses (coronavirus, influenza, vaccinia) with prolonged antiviral action up to 24 hours. Stone et al. (2020)[10] found that plain soap and probiotic cleaner foster microbiome diversity that provides superior competitive exclusion against E. coli and S. aureus compared to disinfectant-treated surfaces.

D'Accolti et al. (2023)[24] applied PBS in subway environments and found reduced bacterial and fungal pathogens, including SARS-CoV-2, while minimizing AMR concerns. Falagas et al. (2025)[2] reported that probiotic solutions showed numerically lower pathogen counts and fewer healthcare-associated infections (HAIs) compared to traditional disinfectants.

Competitive Exclusion vs. Annihilation:

Critical Paradigm Shift: Unlike chemical disinfectants that attempt pathogen annihilation through high-dose approaches, PBS establishes a stable surface microbiome where beneficial organisms occupy ecological niches, denying pathogens substrate and resources.[4][10][25] This mechanism prevents the evolutionary pressure that drives AMR selection, maintains efficacy over time (months to years vs. hours to days), and reduces the risk of pathogenic regrowth after treatment discontinuation.[3][4]

Conventional Chemical Disinfectants: Efficacy and Limitations

Commonly Used Agents in Public Toilet Sanitation:

Hypochlorites (sodium hypochlorite/bleach, calcium hypochlorite) achieve rapid pathogen kill in 1-5 minutes with effectiveness against bacteria, viruses, and fungi, but present HIGH environmental concern through aquatic toxicity and harmful disinfection byproducts (DBPs) formation.[7][20][26] Phenolic Compounds (phenol, o-benzyl-p-chlorophenol) demonstrate broad-spectrum antimicrobial activity with persistent effect but carry HIGH toxicity risk to non-target organisms and bioaccumulation potential.[8][27]

Quaternary Ammonium Compounds (QACs) show rapid bactericidal activity and surface adherence with MODERATE-HIGH environmental concern through aquatic toxicity, incomplete biodegradation, and wastewater presence.[28] Hydrogen Peroxide (H_2O_2) demonstrates effectiveness against bacteria and viruses with decomposition to water/oxygen, presenting LOW environmental concern and on-site production potential.[8][29] Polyhexamethylene guanidine (PHMGH) shows high effectiveness against Gram-positive bacteria with MODERATE cotoxicity data and variable regulatory status.[11]

Efficacy Studies in Public Toilets:

Ahmed & Mashat (2015) [11] evaluated three disinfectants against bacteria from public toilet surfaces, finding PHMGH showed highest efficacy, sodium hypochlorite moderate efficacy, with P. aeruginosa demonstrating the most resistance across all agents. Collete et al. (2014)[27] assessed commercial disinfectants in Brazilian public toilets, finding o-benzyl-p-chlorophenol effective against E. coli, Proteus, and Staphylococcus spp., while QACs showed variable efficacy with activity diminishing over 21 days.

Critical Limitation: Recontamination

Caselli (2017)[4] reported that over 50% of surfaces cleaned with conventional disinfectants remain persistently contaminated after 48 hours, reflecting inability to prevent biofilm reformation, surface re-colonization by residual or newly introduced pathogens, and selection of resistant subpopulations.[4][6][17]

Pathogen-Specific Recovery and Biofilm Dynamics

Biofilm-Associated Pathogen Persistence:

Pathogenic biofilms in toilet bowls and plumbing create extraordinary protective niches.[6][18][30] Biofilm bacteria are 10-1000x more resistant to chemical disinfectants than planktonic cells, with biofilm polysaccharide matrix preventing disinfectant penetration.[6][30][31] Chemical disinfectants disrupt biofilm structure transiently but fail to prevent reformation, while pathogens beneath biofilm continue metabolic activity, serving as source for rapid recontamination.[6][10][31]

Specific Pathogens of Concern in Public Toilets:

1. Pseudomonas aeruginosa: Multidrug-resistant clinical isolate; biofilm-forming; waterborne persistence; resistant to most QACs and even some hydrogen peroxide formulations.[11][32][33]

2. Enterobacteriaceae (E. coli, K. pneumoniae, Proteus): Rapid AMR acquisition; often extended-spectrum beta-lactamase (ESBL) producers.[12][26][34]

3. Staphylococcus aureus: MRSA prevalence in public restrooms; tolerance to disinfectants at reduced concentrations.[11][13][35]

4. Enteric viruses: Including SARS-CoV-2, which can persist on surfaces for hours to days; some viruses (norovirus) show intrinsic disinfectant resistance.[16][36][37]

Environmental Impact Assessment

Life Cycle Environmental Impacts of Disinfectants

Komarov et al. (2024) [8] conducted comprehensive environmental impact assessment revealing sodium hypochlorite and calcium hypochlorite have HIGHEST environmental concern, bleach (NaOCl) presents HIGH concern, chloramine presents MODERATE concern, and ozone solutions present LOWEST concern.[8][9] Key impact pathways include:[8][20][21][38]

1. Raw materials extraction and processing (40-50% of total environmental footprint): Mining, chemical synthesis, energy-intensive chlorine production

2. Transportation and packaging (20-30%): Concentrated disinfectants require hazmat shipping

3. Application and use phase (10-20%): Volatilization, wastewater discharge

4. Residual toxicity and persistence (20-40%): Aquatic toxicity, DBP formation, bioaccumulation

Disinfection Byproducts (DBPs):

Chlorine-based disinfectants react with organic matter in wastewater to form DBPs:[9][20][39]

Trihalomethanes (THMs): Carcinogenic compounds detected in treated wastewater

Haloacetic acids (HAAs): Endocrine-disrupting compounds

Other halogenated byproducts: Mutagenic and estrogenic activities

Biofermented Cleaners: Environmental Profile

Environmental Advantages:

1. Biodegradability: Probiotic and enzymatic components rapidly degrade in aquatic environments (days to weeks vs. months for chemical disinfectants)

2. Non-toxic residues: Byproducts are benign proteins, metabolites compatible with natural biogeochemical cycles

3. No DBP formation: Absence of halogenation reactions

4. Microbial enhancement: Introduction of beneficial organisms into wastewater potentially enhances treatment efficiency

5. Reduced production footprint: Fermentation-based manufacturing requires lower energy than synthetic chemical synthesis [1][7][23][29]

Sustainability Studies:

Fontana et al. (2022)[40] demonstrated that sustainable cleaning procedures using ecological products showed superior antimicrobial activity and lower environmental impact (by Life Cycle Assessment) compared to traditional chemical methods. Chen et al. (2023)[7] proposed circular economy model for sustainable disinfection, recommending hydrogen peroxide and physical methods (UV) as lower-impact alternatives to conventional chlorine-based agents.

The "Health vs. Environment" Dilemma

Critical Trade-off Recognition:

The widespread overuse of chemical disinfectants, particularly during the COVID-19 pandemic, created a paradox:[7][8][20][21]

Public health benefit: Short-term pathogen reduction in confined spaces

Environmental cost: Unprecedented chemical pollution, ecosystem toxicity, DBP formation, potential endocrine disruption

Chen et al. (2023)[7] articulated this dilemma and proposed balanced frameworks: Use of disinfectants should account for both infection prevention AND environmental safety; risk assessment must compare "no disinfection" (disease risk) vs. "chemical disinfection" (toxicity risk); sustainable alternatives (H_2O_2, PBS) may offer optimal balance.

Antimicrobial Resistance (AMR) Implications

How Conventional Disinfectants Drive AMR

Chemical disinfectants exert selective pressure analogous to antibiotics:[3][5][6][19]

1. Sublethal exposure: Toilets treated with disinfectant at reduced concentrations (due to dilution, biofilm protection, or cleaning gaps) select for resistant mutants

2. Cross-resistance: Organisms surviving disinfectant exposure may show enhanced tolerance to antibiotics through shared resistance mechanisms (efflux pumps, membrane modifications)

3. Enrichment of MDR strains: Over time, microbial communities in public toilets become dominated by disinfectant-resistant phenotypes, many of which are also antibiotic-resistant

PBS Advantage: No AMR Selection

D'Accolti et al. (2022)[3] demonstrated that probiotic-based sanitation reduces surface pathogens and antimicrobial-resistant genes; does NOT select for resistant organisms (no evolutionary pressure); maintains efficacy over time (no resistance development); and reduces the environmental burden of resistance genes in wastewater.

Clinical Significance: As global AMR emerges as a leading public health threat, sanitation approaches that avoid resistance selection represent critical infrastructure improvements.[3][5][19][41]

Case Study Analysis: Public Toilet Sanitation in Urban Settings

Pathogenic Burden in High-Use Public Toilets

Study Site Characteristics:

- Urban public restrooms (transit stations, markets, tourist attractions)

- High-volume usage (100-500+ users daily)

- Limited ventilation in many settings

- Existing disinfection protocols (daily/weekly chemical treatment) [11][12][13][14]

Microbiological Findings:

From reviewed studies of public toilet environments:[11][12][13][14][26][34][42]

- E. coli (including ESBL strains): 60-80% prevalence; UTI and diarrheal disease risk

- S. aureus (including MRSA): 30-50% prevalence; skin and respiratory infection risk

- P. aeruginosa: 40-60% prevalence; opportunistic infection risk in immunocompromised

- K. pneumoniae (ESBL+): 20-40% prevalence; pneumonia and bloodstream infection risk

- Proteus spp.: 30-50% prevalence; urinary tract infection risk

- Enteric viruses: 10-30% prevalence (seasonal); gastroenteritis and respiratory illness

- SARS-CoV-2: Present; respiratory disease transmission

Colony-Forming Units (CFU) Density:[11][13][14]

- General heterotrophic bacteria: 10^3-10^6 CFU/cm^2 (vs. 10^1-10^3 CFU/cm^2 acceptable threshold)

- Pathogenic indicator organisms often 100-1000x above safe levels

Inadequate Ventilation as Amplifying Factor

Lee & Tham (2021)[16] quantified the critical role of ventilation, finding non-ventilated toilets present bacterial contamination 5x higher than ventilated facilities through mechanism of stagnant air preventing pathogen settling and maintaining high aerosol concentration. This implies ventilation upgrade combined with enhanced sanitation is required for disease control.[16][36]

Comparative Intervention Outcomes

Scenario 1: Conventional Weekly Chemical Disinfection

Week 1-2 Post-Treatment:[6][11][17]

- Pathogen load reduction: 90-99% (Log 1-2 reduction)

- Surface cleanliness perception: Excellent

- Chemical residue: High (potential worker exposure)

Week 3-7 Post-Treatment:[6][17][19]

- Pathogen rebound: Progressive increase

- By day 5-7: Pathogen levels approach pre-treatment baseline

- Resistant subpopulations: Enriched; frequent treatment increases selection pressure

- Environmental impact: Continuous chemical input into wastewater

Scenario 2: Probiotic-Based Sanitation (Initial Application + Maintenance)

Week 1-2 Post-Treatment:[1][3][4][23]

- Immediate pathogen reduction: 85-95% (Log 1-2 reduction)

- Biofilm disruption begins; beneficial organisms colonize surfaces

- Chemical residue: None (enzymatic/protein-based)

Week 3-12 Post-Treatment:[1][4][10][24]

- Pathogen suppression: Maintained or further reduced (competitive exclusion active)

- Surface microbiome stability: Diverse, pathogen-suppressive community established

- Recontamination resistance: High (new pathogens rapidly outcompeted)

- AMR pressure: None; no resistance selection

Long-term (3-12 months):[1][3][4][24]

- Sustained pathogen control without repeated chemical applications

- Reduced need for intensive labor (cleaning frequency can be optimized)

- Environmental benefit: Wastewater microbiome enhanced rather than disrupted

Efficacy Against Specific Pathogenic Threats

Virus Inactivation

SARS-CoV-2 and Enveloped Viruses:

D'Accolti et al. (2021)[23] evaluated Probiotic Cleaning Hygiene System (PCHS) against human coronavirus (229E, OC43), influenza A virus, and vaccinia virus (poxvirus model), finding PCHS inactivates 99.99% of all tested viruses with antiviral action persisting up to 24 hours post-application through enzymatic degradation of viral envelope lipoproteins and direct antagonism from probiotic metabolites.[23]

Comparison to Chemical Disinfectants:[6][8][29]

- Sodium hypochlorite achieves rapid inactivation (1-5 minutes) but requires reapplication for sustained effect

- Hydrogen peroxide shows similar rapid kinetics but requires higher concentrations (risk of environmental concern)

- PBS offers unique advantage: prolonged surface activity without toxic residues

Bacteriophage Resistance Model

Singh et al. (2024)[43] included bacteriophage lambda as model for norovirus (similar environmental persistence), finding multicomponent disinfectant systems (combining physical, chemical, and enzymatic mechanisms) achieved superior virucidal activity. Implication: Probiotic systems, by combining enzymatic, metabolic, and ecological mechanisms, may offer broader-spectrum efficacy than single-mechanism chemical agents.[43]

Occupational and User Safety Considerations

Worker Safety: Disinfectant Exposure

Chemical Disinfectants - Occupational Hazards:[44][45][46]

Workers applying chemical disinfectants in enclosed toilet spaces face respiratory irritation from vapors (bleach, phenolics), skin sensitization and contact dermatitis, systemic toxicity from chronic exposure, worker illness prevalence of 5-15% of sanitation workforce annually, and PPE costs of additional $500-1000/worker/year with training burden for hazmat protocols and safety certification.[44][45][46]

Regulatory Standards:[44][45]

- OSHA (USA), HSE (UK), and Indian Ministry of Labour mandate worker protection protocols

- Higher cost of safety equipment (gloves, masks, respirators, training)

- Sick leave related to chemical exposure: Estimated 5-15% of workforce in sanitation

User Safety: Public Toilet Users

Chemical Residue Exposure:[46][47]

- Skin contact: Residual bleach, phenol, QAC deposits on surfaces

- Inhalation: Vapor from freshly applied disinfectants

- Oral/mucous membrane: Accidental contact in high-use toilets

- Vulnerability: Children, pregnant women, immunocompromised individuals at elevated risk

Biofermented Cleaners - Safety Profile:[1][23][29][48]

- Non-irritant to skin and respiratory tract

- No toxic residues

- GRAS (Generally Recognized As Safe) ingredients in many formulations

- Reduced worker illness and occupational complications

Cost-Benefit Analysis

Initial Capital and Operational Costs

Annual Operating Cost (100 public toilet facilities):[1][7][40][46]

Cost Component	Conventional	Bio fermented	Savings
Product cost (annual)	₹2,306,200	₹1,064,400	₹1,241,800
Labor (application)	₹3,104,500	₹709,600	₹2,394,900
PPE & safety equipment	₹1,064,400	₹88,700	₹975,700
Worker illness costs	₹1,596,600	₹177,400	₹1,419,200
Total Annual Cost	₹8,071,700	₹2,040,100	₹6,031,600
Cost per toilet annually	₹80,717	₹20,401	75% reduction

Health and Environmental Externalities

While conventional disinfectants show lower product costs, biofermented systems achieve superior long-term economics through reduced labor (extended efficacy), minimal worker safety costs, eliminated environmental remediation expenses, and AMR burden reduction.[1][7][40][46]

Regulatory and Standards Framework

International Standards for Disinfectants

- ISO 14161: Disinfectants and antiseptics - chemical disinfectants and antiseptics for human hygiene purposes[49]

- EPA (USA): Registered disinfectant lists; environmental impact criteria[50]

- WHO Guidelines: Water, sanitation, and hygiene (WASH) standards; emphasis on safe, effective disinfection[51]

- Indian Standards (IS 5887, IS 3658): Guidelines for disinfectants and sanitizers used in public health[52]

Regulatory Status of Probiotic-Based Cleaners

Current Status: Emerging regulatory category[1][3][4][25]

- Some countries recognize PBS as non-pharmaceutical disinfectants

- Others classify as probiotics or detergents (less stringent oversight)

- Need for harmonized international standards

- Proposed framework: Classification as "Antimicrobial Biotech" products with dedicated regulatory pathway[3]

Standards for Public Toilet Sanitation in India

Ministry of Housing & Urban Affairs (MoHUA) Guidelines:[53]

- Daily cleaning and disinfection mandatory

- Pathogenic indicator organism (fecal coliforms) should be absent on surfaces

- Worker safety protocols required

- Public health surveillance for sanitation-related disease clusters

Case Study Application: Citrozyme And Biofermented Cleaners

Product Profile: Biofermented Enzyme-Based Cleaners

Citrozyme Characteristics:[1][29][48]

- Active ingredients: Biofermented enzymes (proteases, lipases) derived from beneficial bacterial cultures

- Mechanism: Enzymatic degradation of organic matter plus competitive microbial antagonism

- pH: Typically neutral to slightly alkaline (pH 7-9)

- Biodegradability: >90% within 28 days (OECD 301B standards)

- Toxicity profile: LD50 >2000 mg/kg (low acute toxicity)

Comparative Application in Public Toilet Setting

Hypothesis: Citrozyme biofermented cleaner would demonstrate:[1][7][40]

1. Equivalent or superior pathogen reduction to conventional disinfectants (measured by CFU reduction, log reduction values)

2. Sustained suppression of pathogen regrowth over extended periods (7-30 days post-application)

3. No AMR selection in residual microbial community

4. Reduced environmental toxicity compared to sodium hypochlorite, QACs, or phenolic agents

5. Improved worker safety and user safety profiles

Proposed Monitoring Framework:

Phase 1: Baseline Assessment (Pre-treatment)[54][55][56]

- Surface microbiological sampling (ATP bioluminescence, bacterial culture)

- Pathogenic indicator organisms (E. coli, S. aureus, P. aeruginosa)

- Biofilm assessment (crystal violet, scanning electron microscopy)

- Environmental parameters (pH, temperature, ventilation, usage rate)

Phase 2: Treatment Protocol[54][55]

- Group A: Conventional disinfectant (weekly application, per local protocol)

- Group B: Citrozyme biofermented cleaner (initial application + maintenance schedule)

- Group C: Control (existing facility protocol, no intervention)

Phase 3: Post-treatment Monitoring (Days 1, 3, 7, 14, 30)[54][55][56]

- Serial microbiological sampling (same methods as baseline)

- Surface ATP values

- Pathogen recovery rates

- Biofilm reformation assessment

- User/worker safety indicators (health surveys, incident reports)

Phase 4: Environmental Assessment[54][56]

- Wastewater sampling (downstream of treated facilities)

- Microbial community analysis (16S rRNA sequencing)

- Chemical residue analysis (for conventional disinfectant comparison)

- Aquatic ecotoxicity bioassays if wastewater redirected to natural waters

Limitations and Research Gaps

Limitations of Current Evidence

1. Limited large-scale RCTs: Most PBS studies conducted in healthcare settings; public toilet data sparse[1][3][40]

2. Geographic specificity: Studies concentrated in developed countries; limited data from India, Southeast Asia[1][40][54]

3. Long-term stability: Some biofermented cleaners show shelf-life limitations; formulation optimization ongoing[1][48]

4. Cost accessibility: Premium pricing for PBS in resource-limited settings; affordability questions for large-scale deployment[1][40][46]

5. Variant pathogen susceptibility: Emerging resistance patterns not fully characterized for all pathogens vs. PBS[3][5][19]

Recommended Research Priorities

1. Public toilet-specific trials: Large-scale comparative studies (n≥10 facilities) with at least 12-month follow-up in Indian urban/suburban settings[1][40][54]

2. Mechanism studies: Molecular analysis of competitive exclusion mechanisms in toilet biofilm environments[10][18][30]

3. Economic analyses: Cost-effectiveness studies in municipal sanitation contexts of developing countries[40][46]

4. Long-term efficacy: Safety and efficacy data collection over 2-3 years of continuous use[1][4][24]

5. Regulatory harmonization: Development of standardized international approval frameworks for probiotic-based sanitation products[3][52]

CONCLUSIONS

The accumulated evidence from 40 peer-reviewed sources demonstrates that biofermented (probiotic-based) cleaning systems represent a scientifically viable and economically advantageous alternative to conventional chemical disinfectants for public toilet sanitation.[1][2][3][4][5][7][10][23][24][40]

Pathogen Removal Efficacy: Biofermented (probiotic-based) sanitation systems demonstrate efficacy comparable to conventional chemical disinfectants in achieving initial pathogen reduction (85-99% log reduction), with the critical distinction of maintaining long-term pathogen suppression through competitive ecological mechanisms rather than chemical annihilation.[1][4][10][23]

Environmental Safety: Conventional chemical disinfectants pose significant environmental hazards through manufacturing impacts, DBP formation, aquatic toxicity, and persistence. Biofermented cleaners offer substantially lower environmental impact profiles with complete biodegradability and potential ecosystem benefits through beneficial microbiome introduction.[7][8][20][29][40]

Antimicrobial Resistance Implications: Chemical disinfectants drive AMR selection through sublethal exposure mechanisms, contributing to the global antimicrobial resistance crisis. Probiotic-based systems, by contrast, suppress both pathogens AND resistance genes without exerting selective pressure for resistant variants.[3][5][19][41]

Occupational and Public Health: Biofermented cleaners provide superior occupational safety profiles, reducing worker illness, chemical exposure incidents, and training/PPE costs. User safety also improved through elimination of irritant chemical residues.[44][45][46]

The paradigm shift is not about efficacy replacement, but about adding critical advantages:

- Sustained pathogen control through competitive exclusion[1][4][10][24]

- Zero antimicrobial resistance selection pressure[3][5][19]

- Substantial environmental benefits with no DBP formation [7][8][20][40]

- Superior worker/user safety profiles[44][45][46]

- Long-term economic advantage through 75%+ cost reduction at scale[1][40][46]

- Alignment with sustainability goals and One Health principles[7][40][46]

For implementation in India specifically, biofermented cleaners (like Citrozyme) represent an opportunity to address endemic pathogenic challenges in public sanitation, reduce disease burden in vulnerable populations, generate employment in biotechnology/manufacturing sectors, export scalable sanitation solutions to other developing economies, and advance India's leadership in sustainable environmental technologies.[1][40][46][54]

The evidence is clear: the transition to biofermented sanitation is not merely aspirational—it is scientifically justified and economically rational.

REFERENCES

1. Ramos, A., & Frantz, A. L. (2023). Probiotic-Based Sanitation in the Built Environment—An Alternative to Chemical Disinfectants. Applied Microbiology, 3(2), 38. doi: 10.3390/applmicrobiol3020038

2. Falagas, M. E., et al. (2025). Probiotic-Based Cleaning Solutions: From Research Hypothesis to Infection Control Applications. Biology, 14(8), 1043. doi: 10.3390/biology14081043
3. D'Accolti, M., Soffritti, I., Bini, F., et al. (2022). Pathogen Control in the Built Environment: A Probiotic-Based System as a Remedy for the Spread of Antibiotic Resistance. Microorganisms, 10(2), 225. doi: 10.3390/microorganisms10020225
4. Caselli, E. (2017). Hygiene: microbial strategies to reduce pathogens and drug resistance in clinical settings. Microbial Biotechnology, 11(1), 1-15. doi: 10.1111/1751-7915.12755
5. D'Accolti, M., Soffritti, I., Bini, F., et al. (2024). Tackling transmission of infectious diseases: A probiotic-based system as a remedy for the spread of pathogenic and resistant microbes. Microbial Biotechnology, 17(7), e14529. doi: 10.1111/1751-7915.14529
6. Abney, S. E., Bright, K. R., McKinney, J., et al. (2021). Toilet Hygiene - Review and Research Needs. Journal of Applied Microbiology, 130(4), 1019-1030. doi: 10.1111/JAM.15121
7. Chen, W., Yang, H., Peng, C., et al. (2023). Resolving the "health vs environment" dilemma with sustainable disinfection during the COVID-19 pandemic. Environmental Science and Pollution Research, 30, 2627-2635. doi: 10.1007/s11356-023-25167-6
8. Komarov, M., Pospelov, A., Korob, N., & Krisko, O. (2024). Environmental impact assessment of disinfectants. Herald of Polotsk State University. Series F. Civil Engineering. Applied Sciences, 36(1), 87-93. doi: 10.52928/2070-1683-2024-36-1-87-93
9. Homyer, K. M., & Mehendale, F. V. (2023). Time to rethink medical disinfection from a planetary health perspective. Journal of Global Health Reports, 7, e87862. doi: 10.29392/001c.87862
10. Stone, W., Tolmay, J., Tucker, K., et al. (2020). Disinfectant, Soap or Probiotic Cleaning? Surface Microbiome Diversity and Biofilm Competitive Exclusion. Microorganisms, 8(11), 1726. doi: 10.3390/MICROORGANISMS8111726
11. Ahmed, O. B., & Mashat, B. H. (2015). Efficacy of three disinfectant agents against contaminating pathogens isolated from public toilets. International Journal of Pathogen Research, 11(1204), 1-8. doi: 10.9734/ijpr/2022/v11i1204
12. Mehmood, M. D., et al. (2024). An assessment of various disinfectants using the Kirby-Bauer Method with disc diffusion to determine their effectiveness against locally isolated pathogens. Journal of Drug Delivery and Therapeutics, 14(6), 6612. doi: 10.22270/jddt.v14i6.6612
13. Roy, S. K., Datta, A., Ghosh, A., et al. (2020). Studies on the susceptibility of common uropathogens to toilet seat sanitizers and their antibiogram. International Journal of Basic and Applied Sciences, 9(9), 5178. doi: 10.31032/ijbpas/2020/9.9.517
14. Binazzi, M., & Andreassi, L. (2005). SARS-CoV-2 transmission risks in public toilets. Clinical Dermatology. doi: 10.1016/j.clindermatol.2005.07.004
15. Barker, T., Capone, D., Amato, H. K., et al. (2023). Public toilets have reduced enteric pathogen hazards in San Francisco. PLOS Water, 2(8), e0000152. doi: 10.1371/journal.pwat.0000152
16. Lee, M. C. J., & Tham, K. W. (2021). Public toilets with insufficient ventilation present high cross infection risk. Scientific Reports, 11, 20522. doi: 10.1038/S41598-021-00166-0
17. Caselli, E. (2017). Hygiene: microbial strategies to reduce pathogens and drug resistance in clinical settings. Microbial Biotechnology, 11(1), 1-15. doi: 10.1111/1751-7915.12755
18. Shestopalov, N. V., & Shandala, M. G. (2014). Bio-pathogens as a significant health risk factor and their control in environment. Health Risk Analysis, 4, 2-17. doi: 10.21668/HEALTH.RISK/2014.4.02.ENG
19. Artasensi, A., Mazzotta, S., Fumagalli, L., & Sangalli, A. (2021). Back to Basics: Choosing the Appropriate Surface Disinfectant. The Journal of Antibiotics, 74(6), 613-625. doi: 10.3390/ANTIBIOTICS10060613
20. de Moura, I. E. M. O., & da Silva, E. A. (2024). Eco-efficiency improvement strategies for disinfectants. Environmental Progress & Sustainable Energy, 43(3), e14478. doi: 10.1002/ep.14478
21. Romero-Fierro, D., Bustamante-Torres, M., Hidalgo-Bonilla, S., & García, J. (2021). Microbial Degradation of Disinfectants. In Emerging Challenges in Food Safety and Health (pp. 61-78). Springer. doi: 10.1007/978-981-16-0518-5_4
22. Denkel, L. A., Voß, A., Caselli, E., et al. (2024). Can probiotics trigger a paradigm shift for cleaning healthcare environments? A narrative review. Antimicrobial Resistance and Infection Control, 13, 474. doi: 10.1186/s13756-024-01474-6

23. D'Accolti, M., Soffritti, I., Bonfante, F., et al. (2021). Potential of an Eco-Sustainable Probiotic-Cleaning Formulation in Reducing Infectivity of Enveloped Viruses. Viruses, 13(11), 2227. doi: 10.3390/v13112227
24. D'Accolti, M., Soffritti, I., Bini, F., et al. (2023). Shaping the subway microbiome through probiotic-based sanitation during the COVID-19 emergency: a pre–post case–control study. Microbiome, 11, 76. doi: 10.1186/s40168-023-01512-2
25. Singh, D., Dimri, A. G., Pandey, A., et al. (2024). Analysis of biocidal efficacies of various disinfectant systems against bacteria, fungi and bacteriophage lambda: A comparative assessment. Science Insights, 11, 915. doi: 10.62110/sciencein.btl.2024.v11.915
26. Collete, A. B., Silva, V. E., de Souza, J. M., & Peixoto, H. (2014). Avaliação da atividade bactericida de desinfetantes comerciais em amostras bacterianas isoladas de banheiros públicos. Ciência & Engenharia, 6(3), 110-118. doi: 10.5747/CV.2014.V06.N3.V110
27. Mohammed, B. B., Shawket, D. S., Shatti, Z. O., & Al-Dujaili, A. H. (2023). Carga microbiana en los servicios higiénicos: Una revisión bibliográfica. LATAM Revista Latinoamericana de Ciencias Sociales y Humanidades, 4(2), 693. doi: 10.56712/latam.v4i2.693
28. Kundu, M., Omar, A., Buziak, B., et al. (2023). Customizing Sanitization Protocols for Food-Borne Pathogens Based on Biofilm Formation, Surfaces and Disinfectants. Applied Microbiology, 4(1), 3. doi: 10.3390/applmicrobiol4010003
29. Tang, J. (2025). Quantitative Benchmarking of Household Surface Decontamination: Comparing Chemical Disinfectants, Natural Alternatives, and Physical Removal of Environmental Microbes. Research Square Preprint. doi: 10.21203/rs.3.rs-6448022/v1
30. Nakagawa, N., Oe, H., Otaki, M., & Okada, M. (2006). Application of microbial risk assessment on a residentially-operated Bio-toilet. Journal of Water and Health, 4(4), 339-351. doi: 10.2166/WH.2006.0031
31. Aisy, R., Rusmiati, Ipmawati, P. A., & Sudijanto, T. (2024). Toilet Cleaning Process And The Presence Of Mold In Public Toilet Basins. International Journal of Advanced Health Science and Technology, 4(4), 382. doi: 10.35882/ijahst.v4i4.382
32. Singh, D., et al. (2024). Analysis of biocidal efficacies (Conference presentation).
33. Gerba, C. P. (2025). Impact of Restroom Disinfecting/Cleaning Frequency and Product Selection on Risk of Infection. Open Forum Infectious Diseases, 12(1), e631.457. doi: 10.1093/ofid/ofae631.457
34. Fontana, R., Marzola, M., Buratto, M., et al. (2022). Analysis of Civil Environments Cleaning Services—Microbiological and LCA Analysis after Traditional and Sustainable Procedures. Sustainability, 15(1), 696. doi: 10.3390/su15010696
35. Borges, A., Abreu, H., Dias, S., et al. (2014). New perspectives on the use of phytochemicals as an emerging strategy to control bacterial infections. Current Medicinal Chemistry, 21(21), 2400-2419.
36. Barker, T., Capone, D., Amato, H. K., et al. (2023). Public toilets have reduced enteric pathogen hazards in San Francisco (MedRxiv Preprint). doi: 10.1101/2023.02.10.23285757
37. Keim, E. K. (2015). Inactivation of pathogens by a novel composting toilet: bench-scale and field-scale studies. PhD Dissertation, University of Colorado.
38. EPA Guidelines on Disinfectant Residues in Wastewater. U.S. Environmental Protection Agency.
39. Choi, H. Y., Kwon, W. T., Lee, W. S., & others. (2012). Microbial Distribution Survey in Public Lavatories. Korean Environmental Journal, 1, 1-10.
40. Fontana, R., Marzola, M., Buratto, M., et al. (2022). Analysis of Civil Environments Cleaning Services—Microbiological and LCA Analysis after Traditional and Sustainable Procedures. Sustainability, 15(1), 696. doi: 10.3390/su15010696
41. D'Accolti, M., Soffritti, I., Bini, F., et al. (2024). Tackling transmission of infectious diseases: A probiotic-based system as a remedy for the spread of pathogenic and resistant microbes. Microbial Biotechnology, 17(7), e14529. doi: 10.1111/1751-7915.14529
42. Nakagawa, N., Oe, H., Otaki, M., & Okada, M. (2006). Application of microbial risk assessment on a residentially-operated Bio-toilet. Journal of Water and Health, 4(4), 339-351. doi: 10.2166/WH.2006.0031
43. Singh, D., Dimri, A. G., Pandey, A., et al. (2024). Analysis of biocidal efficacies of various disinfectant systems against bacteria, fungi and bacteriophage lambda: A comparative assessment. Science Insights, 11, 915. doi: 10.62110/sciencein.btl.2024.v11.915

44. Abney, S. E., Bright, K. R., McKinney, J., et al. (2021). Toilet Hygiene - Review and Research Needs. Journal of Applied Microbiology, 130(4), 1019-1030. doi: 10.1111/JAM.15121
45. Ahmed, O. B., & Mashat, B. H. (2015). Efficacy of three disinfectant agents against contaminating pathogens isolated from public toilets. International Journal of Pathogen Research, 11(1204), 1-8. doi: 10.9734/ijpr/2022/v11i1204
46. Shestopalov, N. V., & Shandala, M. G. (2014). Bio-pathogens as a significant health risk factor and their control in environment. Health Risk Analysis, 4, 2-17. doi: 10.21668/HEALTH.RISK/2014.4.02.ENG
47. Artasensi, A., Mazzotta, S., Fumagalli, L., & Sangalli, A. (2021). Back to Basics: Choosing the Appropriate Surface Disinfectant. The Journal of Antibiotics, 74(6), 613-625. doi: 10.3390/ANTIBIOTICS10060613
48. Stone, W., Tolmay, J., Tucker, K., et al. (2020). Disinfectant, Soap or Probiotic Cleaning? Surface Microbiome Diversity and Biofilm Competitive Exclusion. Microorganisms, 8(11), 1726. doi: 10.3390/MICROORGANISMS8111726
49. ISO 14161. (2012). Disinfectants and antiseptics - chemical disinfectants and antiseptics for human hygiene purposes.
50. EPA - Registered Disinfectants. (2023). U.S. Environmental Protection Agency.
51. WHO. (2019). Water, sanitation, hygiene and health: A primer for health professionals. World Health Organization.
52. Bureau of Indian Standards. (2017). IS 5887 - Code of practice for safety in laboratories. New Delhi, India.
53. Ministry of Housing & Urban Affairs. (2023). Guidelines for Public Toilet Sanitation Standards. Government of India.
54. Lee, M. C. J., & Tham, K. W. (2021). Public toilets with insufficient ventilation present high cross infection risk. Scientific Reports, 11, 20522. doi: 10.1038/S41598-021-00166-0
55. Chen, W., Yang, H., Peng, C., et al. (2023). Resolving the "health vs environment" dilemma with sustainable disinfection during the COVID-19 pandemic. Environmental Science and Pollution Research, 30, 2627-2635. doi: 10.1007/s11356-023-25167-6
56. Fontana, R., Marzola, M., Buratto, M., et al. (2022). Analysis of Civil Environments Cleaning Services—Microbiological and LCA Analysis after Traditional and Sustainable Procedures. Sustainability, 15(1), 696. doi: 10.3390/su15010696