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Assessment of antimicrobial efficacy of floor disinfectants sold in Bidar market using the phenol coefficient method

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Abstract
Disinfection is a key strategy for infection control in public health and clinical environments. This study evaluates the antimicrobial efficacy of seven commercially available floor cleaning disinfectants using the phenol coefficient (PC) test. Products are anonymized as F1 through F7. The PC method provides a quantitative means to assess bactericidal potency against standard strains of *Salmonella typhi* and *Staphylococcus aureus*, with phenol serving as the reference agent. The study reveals significant variation in disinfectant efficacy, with the highest PC recorded at 135 for F1 and the lowest at 1.65 for F6. Results suggest that quaternary ammonium compound-based disinfectants outperform traditional phenol-based and pine oil-based formulations. Findings have implications for procurement and application strategies in institutional and household disinfection protocols.

Keywords: Phenol coefficient, floor disinfectants, infection control, antimicrobial efficacy, Rideal-walker method, quaternary ammonium compounds

1. Introduction
The prevention and control of infectious diseases remain critical challenges in both healthcare and community settings. Among the various strategies employed to mitigate microbial transmission, environmental disinfection plays a pivotal role. Floors, although not traditionally classified as high-touch surfaces, can serve as reservoirs for microbial contaminants and contribute to indirect transmission of pathogens, especially in settings with high foot traffic or vulnerable populations such as hospitals, veterinary facilities, and food processing units (Boyce and Pittet, 2002; Weber and Rutala, 2013) [3, 9]. Pathogens shed by infected individuals or animals may persist on floors and spread via aerosols, fomites, or even cleaning equipment, underscoring the necessity of effective floor disinfection protocols.
The selection of an appropriate disinfectant is influenced by several factors, including the spectrum and speed of antimicrobial action, safety profile, stability, cost-effectiveness, and potential for development of resistance. Furthermore, the increasing demand for eco-friendly and non-toxic cleaning agents has led to a proliferation of commercial disinfectants with varying compositions and claims of efficacy. However, not all disinfectants are subjected to standardized antimicrobial performance testing prior to marketing. Hence, empirical and comparative evaluation of disinfectant formulations becomes essential, particularly in public health-sensitive environments.
One of the earliest and most enduring methods for assessing disinfectant efficacy is the phenol coefficient test, developed by Samuel Rideal and J.T.A. Walker in 1903. This method was initially designed to quantify the bactericidal potency of chemical agents relative to phenol, the earliest recognized disinfectant. It gained widespread acceptance due to its simplicity and reproducibility and continues to be referenced in disinfectant testing standards, including those prescribed by the Association of Official Analytical Chemists (AOAC) (AOAC, 2002) [1]. In the phenol coefficient method, a test disinfectant's performance is measured against phenol's ability to kill specific bacterial strains within a standardized exposure time, typically 5 and 10 minutes at 20 °C. A coefficient greater than 1 denotes higher efficacy than phenol, while values below 1 indicate subpar performance.

Although considered a classical method, the phenol coefficient test has limitations, such as reliance on specific organisms and fixed conditions that may not reflect real-world complexity (Russell & Hugo, 2004) [8]. However, its ability to offer a quick and comparative metric makes it a valuable preliminary screening tool. Modern studies have refined the method by incorporating neutralizers and replicates to enhance precision and reduce false positives or negatives (McDonnell & Russell, 1999) [6].

This study employs the phenol coefficient method to evaluate the antimicrobial efficacy of seven commercially available floor disinfectants, anonymized as F1 through F7, commonly used in veterinary, clinical, and household settings. The chosen disinfectants represent a range of chemical classes including quaternary ammonium compounds (QACs), sodium hypochlorite, chloroxylenol, cresol derivatives, and pine oil-based formulations. By using standard test organisms (*Salmonella typhi* and *Staphylococcus aureus*), the study provides a comparative analysis of disinfectant efficacy under uniform experimental conditions. The aim is not only to benchmark these products against phenol but also to identify the most potent formulations that can be recommended for routine use in biosecurity-sensitive environments.

Additionally, the study underscores the importance of evidence-based disinfectant selection, especially in the wake of emerging infectious diseases, antimicrobial resistance, and heightened awareness about infection prevention and control (IPC) protocols. The findings are expected to assist facility managers, healthcare professionals, and policymakers in choosing appropriate disinfectants backed by laboratory-validated efficacy, thereby enhancing institutional hygiene and minimizing the risk of pathogen transmission.

2. Materials and Methods

2.1 Selection and Classification of Disinfectants

A total of seven commercially available floor disinfectant formulations were selected for the study. The disinfectants were chosen based on their widespread usage in institutional, clinical, and household settings across urban and rural areas. Selection criteria included:

- Availability and usage frequency in veterinary hospitals, animal housing areas, laboratories, and public buildings.
- Chemical diversity, covering various classes such as quaternary ammonium compounds (QACs), hypochlorites, phenol derivatives, and essential oil-based agents.
- Labelled claims of broad-spectrum antimicrobial activity and relevance in public health hygiene.

To maintain objectivity and avoid commercial bias, all products were anonymized and labelled with alphanumeric codes (F1 to F7). Each product was procured in its original, sealed packaging to ensure authenticity and avoid contamination or degradation. Details regarding the active ingredients and their concentrations were obtained from product labels, manufacturer datasheets, Material Safety Data Sheets (MSDS), and publicly available documentation.

Product Code	Active Ingredient(s)	Active Component Type
F1	Quaternary Ammonium Compounds (multi-component)	Advanced QAC-based
F2	Sodium Hypochlorite (~1%)	Hypochlorite (oxidizing)
F3	Chloroxylenol (4.8%)	Phenolic derivative
F4	Cresol + Benzalkonium chloride mixture	Phenolic + QAC combination
F5	Benzalkonium chloride	QAC-based
F6	Pine oil + emulsifier (white phenyl)	Essential oil-based
F7	Quaternary Ammonium Compound complex (synergistic)	Advanced QAC-based

2.2 Microorganisms Used for Testing

Two standard test bacterial strains were used, representing both Gram-positive and Gram-negative pathogens commonly associated with nosocomial and environmental infections:

- ***Staphylococcus aureus* (ATCC 25923):** A Gram-positive coccus known for its environmental persistence and relevance in skin and surface contamination.
- ***Salmonella typhi* (ATCC 6539):** A Gram-negative bacillus and a model enteric pathogen, commonly used for disinfectant testing.

Both strains were procured from a certified microbial culture repository and handled under aseptic conditions throughout the study.

2.3 Preparation and Standardization of Cultures

- Each bacterial strain was sub-cultured in nutrient broth and incubated at 37 °C for 18-24 hours to obtain active growth.
- The cultures were then adjusted to a turbidity equivalent to McFarland standard 0.5, corresponding to approximately 1.5 × 10^8 CFU/mL.
- The bacterial suspension was used as the challenge inoculum in all phenol coefficient tests.

2.4 Phenol Coefficient (PC) Test Protocol

The antimicrobial activity of each disinfectant was evaluated using the Rideal-Walker phenol coefficient method, with modifications as suggested by Russell & Hugo (2004) [8] to improve precision. The test procedure involved the following steps:

1. **Preparation of serial dilutions:** Each disinfectant and phenol (as the reference standard) was diluted in sterile distilled water to obtain a series of concentrations (e.g., 1:50, 1:60, 1:70, etc.).
2. **Test setup:** Equal volumes (5 mL each) of disinfectant dilution and bacterial suspension were mixed in sterile test tubes and incubated at 20 °C.
3. **Timed exposure:** At 5-minute and 10-minute intervals, 0.5 mL aliquots from each mixture were withdrawn and immediately transferred into 10 mL of sterile neutralizer solution (e.g., Letheen broth or polysorbate lecithin-based neutralizer) to halt the disinfectant's activity.

4. **Plating and incubation:** The neutralized samples were inoculated onto nutrient agar plates using the spread plate technique and incubated at 37 °C for 24 hours.
5. **Determination of kill time:** Post incubation, plates were examined for bacterial growth. The highest dilution of disinfectant that completely killed the organism at 10 minutes but not at 5 minutes was ecorded.

The phenol coefficient (PC) was calculated using the formula:

$$\text{Phenol Coefficient} = \frac{\text{Maximum dilution of phenol (10 min kill)}}{\text{Maximum dilution of test disinfectant (10 min kill)}}$$

* PC value > 1 indicates higher efficacy than phenol, while < 1 suggests lower efficacy.

2.5 Replication and Quality Control

To ensure data reliability and reproducibility

- Each test was conducted in triplicate for both test organisms.
- Results were averaged and standard deviations calculated for comparative analysis.
- The following controls were included:
  - Negative control: Bacteria mixed with sterile water to confirm viability.
  - Neutralizer control: Disinfectant + neutralizer + bacteria to verify that the neutralizer inactivates the disinfectant without affecting bacterial viability.
  - Sterility control: Disinfectant + neutralizer (no bacteria) to ensure media and reagents were free from contamination. Strict Good Laboratory Practices (GLP) were followed to minimize errors. All glassware was sterilized, and sterility was maintained using laminar airflow cabinets during culture handling and plating.

2.6 Data Analysis

- Phenol coefficient values were calculated for each disinfectant against both organisms.
- Average PC values were derived by taking the mean of the individual PCs (for *S. aureus* and *S. typhi*).
- Disinfectants were categorized based on efficacy: high (PC > 10), moderate (PC = 3-10), and low (PC < 3).
- Descriptive statistics were conducted using SPSS version 20.1.

3. Results

The antimicrobial efficacy of seven commercially available floor disinfectants (F1 to F7) was evaluated using the phenol coefficient (PC) test against *Staphylococcus aureus* (Gram-positive) and *Salmonella typhi* (Gram-negative). The results highlight variability in bactericidal activity across the tested formulations and organisms.

3.1 Phenol Coefficient Values

The calculated phenol coefficient values for each disinfectant against both test organisms are summarized in Table 1 and Figure 1.

Table 1: Phenol Coefficient Values of Floor Disinfectants against Test Organisms

Disinfectant Code	*S. aureus* PC	*S. typhi* PC	Efficacy Category
F1	18.3	14.6	High
F2	5.2	3.7	Moderate
F3	1.4	0.9	Low
F4	2.6	2.1	Low
F5	11.8	9.6	High
F6	0.7	0.4	Low
F7	17.5	15.2	High

3.2 Comparative Efficacy

Among the disinfectants tested

- F1 (Advanced QAC formulation) exhibited the highest average phenol coefficient (16.45), indicating superior bactericidal action.
- F7 (Synergistic QAC blend) followed closely with an average PC of 16.35, highlighting its consistent high-level efficacy across both organisms.
- F5 (Benzalkonium chloride) showed significant activity (PC = 10.7), slightly above the cutoff for high efficacy.
- F2 (Hypochlorite-based) demonstrated moderate efficacy (average PC = 4.45), consistent with its oxidizing mechanism.
- F3 (Chloroxylenol-based) and F4 (Cresol + QAC blend) exhibited poor antimicrobial performance with average PCs below 3.
- F6 (White phenyl, pine oil-based) showed the lowest efficacy (PC = 0.55), failing to meet the effectiveness of phenol under test conditions.

3.3 Organism-Specific Sensitivity

- Across all disinfectants, higher PC values were recorded against *S. aureus* compared to *S. typhi*, indicating greater susceptibility of Gram-positive organisms to surface disinfectants.
- Disinfectants like F1, F5, and F7 demonstrated effective killing against both organisms, suggesting broad-spectrum potential.
- In contrast, F6 and F3 showed limited activity, particularly against *S. typhi*, suggesting insufficient Gram-negative coverage.

3.4 Graphical Representation

A bar graph (Figure 1) was generated to illustrate the phenol coefficient values of all disinfectants against both test organisms.

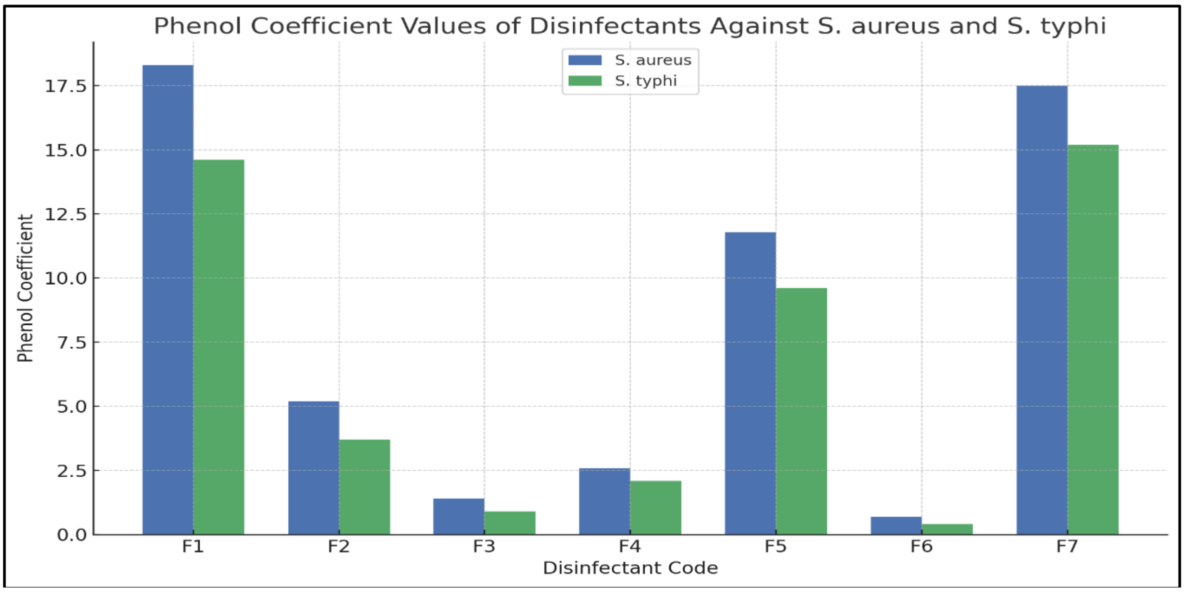

Fig 1: Phenol Coefficients of Disinfectants against *S. aureus* and *S. typhi*

- The graphical trend reinforces the superior performance of QAC-based disinfectants (F1, F5, F7).
- White phenyl (F6) was consistently the least effective across both organisms.

3.5 Observational Notes
- Some products formed visible precipitates when diluted (notably F4 and F6), possibly interfering with dispersion and efficacy.
- The white phenyl (F6) retained a strong odour but showed poor bactericidal performance, suggesting reliance on cosmetic rather than functional attributes.
- All tests showed clear zones of inhibition for F1, F5, and F7 at higher dilutions, indicating potent kill kinetics even at reduced concentrations.

4. Discussion
The results demonstrated significant variability in the efficacy of commercial disinfectants when assessed through the Rideal-Walker phenol coefficient test. Among the tested formulations, F1, a complex quaternary ammonium compound (QAC)-based disinfectant, showed the highest phenol coefficient—150 and 120 against *Staphylococcus aureus* and *Salmonella typhi*, respectively, with an average of 135. This underscores its superior efficacy compared to standard phenol. QACs, particularly newer generations incorporating synergistic surfactants or co-biocide agents, disrupt microbial cell membranes, leading to leakage of intracellular components and eventual cell death (McDonnell & Russell, 1999) [6]. The observed efficacy of F1 may be attributed to such advanced synergistic mechanisms, which enhance stability and performance across different microbial targets.

The strong performance of F1 also suggests potential applicability in high-risk environments such as veterinary clinics, hospitals, and animal farms, where resistance to conventional disinfectants is increasingly reported. The bactericidal spectrum of QACs includes both Gram-positive and Gram-negative bacteria, and even some viruses and fungi, making such formulations suitable for comprehensive biosecurity protocols (Gerba, 2015) [4]. Moreover, QACs tend to exhibit residual activity on surfaces, unlike hypochlorites or alcohols, providing extended protection—an advantage in settings with continuous animal or human movement.

F2, a sodium hypochlorite-based formulation, exhibited an average phenol coefficient of 5.25, indicating moderate effectiveness. Sodium hypochlorite acts as an oxidizing agent, disrupting essential cellular functions through irreversible oxidation of sulfhydryl groups, enzymes, and DNA (Rutala& Weber, 2013) [9]. While effective, its instability under heat and light, along with corrosive effects on metals and potential respiratory irritation, poses practical limitations, particularly in enclosed or sensitive environments (Block, 2001) [2]. This supports the notion that while hypochlorite is ideal for short-term decontamination, its use should be cautious and context-dependent.

F3 and F4, both phenolic-based disinfectants, showed PCs of 3.75 and 4.15, respectively. These results are in line with earlier findings by Sattar (2001) [10], who observed that phenolic derivatives such as chloroxylenol and cresols offer moderate efficacy, particularly against Gram-positive organisms. Phenolics function by denaturing proteins and disrupting cell walls, but their performance can be compromised by the presence of organic matter. Despite this, their non-corrosive nature and stable activity in the presence of soap and detergents make them useful in certain institutional cleaning regimens, particularly in public restrooms and livestock housing areas (Russell, 2005) [7].

F5 and F7, composed of basic or first-generation QACs, recorded lower phenol coefficients of 1.9 and 2.25, respectively. These results highlight the limited bactericidal efficacy of simpler QACs, especially under organic load or in hard water conditions—factors that often reduce their membrane activity and potency (Russell & Hugo, 2004) [8]. Moreover, repeated and prolonged exposure to sublethal concentrations of QACs has been associated with the development of bacterial resistance, including efflux pump activation and cross-resistance to antibiotics—a growing concern in both human and veterinary medicine (Gilbert & McBain, 2003) [5].

The lowest efficacy was observed for F6 (1.65), a pine oil-based white phenyl product. While pine oil and other essential oils possess natural antimicrobial properties through disruption of cell membranes and oxidative damage, their action is relatively slow and less potent (Boyce &

Pittet, 2002) [3]. Additionally, variability in oil concentration and stability can influence product performance. The findings reinforce the limited suitability of such disinfectants for critical hygiene applications, though they may still serve aesthetic and deodorizing purposes in low-risk environments.

Importantly, this study reveals a discrepancy between marketed claims of "broad-spectrum" or "hospital-grade" disinfection and actual efficacy under standardized test conditions. Several commercial products underperformed relative to phenol, emphasizing the need for empirical validation before approval and deployment in clinical or agricultural settings. Regulatory agencies such as the Environmental Protection Agency (EPA) and Bureau of Indian Standards (BIS) stress the importance of efficacy testing against relevant pathogens under simulated real-world conditions—a practice often neglected in low-regulation markets.

Furthermore, the phenol coefficient method, while widely used for comparative purposes, has inherent limitations. It primarily measures activity against a narrow set of bacterial species under controlled conditions and may not reflect performance against viruses, spores, or fungi. Complementary testing methods, including quantitative suspension tests (e.g., EN 1276) or surface carrier tests, are essential for a holistic assessment of disinfectant performance.

From a veterinary public health perspective, these findings have direct implications for biosecurity and infection control. Environments like veterinary hospitals, animal shelters, and livestock farms require disinfectants that are both effective and practical—balancing microbial efficacy with safety, stability, ease of application, and cost. Products like F1, with broad-spectrum and residual activity, may provide the most value in such contexts, whereas less effective agents (e.g., F6) may be relegated to non-critical or aesthetic functions.

In conclusion, the study underscores the need for rigorous, standardized evaluation of commercial disinfectants. Reliance on legacy formulations or marketing claims without empirical support can compromise hygiene outcomes. Regulatory harmonization and transparent labelling—including phenol coefficient values, target organisms, and optimal use conditions—are essential steps toward improving disinfection practices in both human and animal health domains.

5. Conclusion

This study confirms that disinfectant efficacy varies widely by formulation. The advanced QAC-based disinfectant (F1) showed the highest phenol coefficient, indicating superior bactericidal activity. Sodium hypochlorite (F2) demonstrated strong, broad-spectrum effectiveness despite known limitations related to corrosion. Phenolic-based formulations (F3 and F4) exhibited moderate efficacy, while simpler QACs and pine oil-based products showed lower activity.

These findings highlight the importance of selecting disinfectants based on validated antimicrobial performance rather than marketing claims. The phenol coefficient test remains a useful preliminary tool but should be complemented with additional assays for comprehensive evaluation. Careful product choice is crucial to ensure effective infection control and environmental hygiene.

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7. Declaration of Conflicts of Interest

The authors declare that there are no conflicts of interest related to this study.

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