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Original Article

Evaluating the Differential Efficacy of Disinfectants Against Microbial Forms Using Logarithmic Reduction Analysis

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ABSTRACT

Background: Effective disinfection protocols are crucial in healthcare and pharmaceutical settings to mitigate infection and cross-contamination risks, especially with a growing immunocompromised population. Disinfectant efficacy varies, and understanding microbial resistance profiles is essential. This study aimed to evaluate the differential efficacy of ethanol (ET), isopropyl alcohol (IPA), and a peracetic acid-hydrogen peroxide (PA+HP) blend against diverse microbial forms (bacterial spores, fungal spores, and vegetative yeast cells) and to analyze differences in susceptibility.

Methods: The efficacy of ET, IPA, and PA+HP was evaluated against *Bacillus subtilis* (bacterial spores), *Aspergillus niger* (fungal spores), *Candida albicans*, and *Kocuria rosea* (vegetative yeast cells). Logarithmic reduction (LR) values from 10 replicates per group were analyzed using non-parametric (Friedman test with Dunn's post-hoc) and two-way omnibus tests.

Results: The Friedman test revealed significant differences across microbial groups ($p < 0.0001$). *B. subtilis* showed maximal susceptibility (LR = 6.70 ± 0.00), while *A. niger* exhibited minimal susceptibility (LR = 3.77 ± 0.21). ET outperformed IPA against *C. albicans* (LR = 5.43 vs. 4.91, $p = 0.0232$). The microbial group accounted for 92.36% of the variance ($p < 0.0001$).

Conclusions: Microorganism-specific disinfection strategies were emphasized by the findings. A routine disinfectant evaluation program is crucial to mitigate microbial infection and cross-contamination risk in healthcare settings. The study highlights the importance of selecting appropriate disinfectants based on microbial resistance profiles.

Key words: Disinfectant efficacy, microbial resistance, ethanol, isopropyl alcohol, logarithmic reduction

INTRODUCTION

Disinfectant efficacy was observed to vary significantly across microbial forms due to structural and metabolic differences. Bacterial spores (e.g., *Bacillus subtilis*) and fungal spores (e.g., *Aspergillus niger*) exhibit heightened resistance compared to vegetative cells, necessitating tailored disinfection strategies. [1–3] Alcohols like ethanol (ET) and isopropyl alcohol (IPA) are widely used, but their performance against diverse microorganisms remains understudied. [4] It is important to perform a study that combines statistical methods to analyze Logarithmic reduction (LR) values, ensuring robustness against non-normality and outliers. [5] Microbial contamination poses significant risks to public health, necessitating robust disinfection protocols. To mitigate the risk of microbial infection and contamination, various techniques have been adopted to assess and verify the ability of the antimicrobial formulae against test microorganisms, including the

challenging surface tests. [4] Bacterial spores (e.g., *Bacillus subtilis*) and fungal spores (e.g., *Aspergillus niger*) exhibit heightened resistance due to structural adaptations like keratinized coats and antioxidant enzymes. [2,3] Conversely, vegetative cells (e.g., *Candida albicans*) are more susceptible to chemical agents such as alcohols. [5] LR, defined as the $\log_{10}$ reduction in viable microbial counts post-treatment, is a standardized metric for disinfectant efficacy. [6] Regulatory bodies often mandate LR thresholds; for example, a 6-LR is required for high-level disinfection in healthcare settings. [7] Despite this, comparative studies on disinfectant performance across diverse microbial forms remain limited. In disinfectant efficacy studies, the selection of test microorganisms is critical to accurately assess the broad-spectrum activity of the disinfectant against relevant microbial groups encountered in real-world settings. [7]

Bacillus subtilis

This bacterium is frequently included in disinfectant studies because it is a well-established model organism for testing sporicidal activity. *Bacillus spp.* is known to form highly resistant endospores that can withstand harsh environmental conditions, including many disinfection processes. Evaluating a disinfectant's efficacy against *B. subtilis* spores provides crucial information about its ability to inactivate resilient microbial forms that are challenging to eliminate, making it highly relevant for ensuring effective sterilization or high-level disinfection. [8,9]

Aspergillus niger

As a common environmental mold, *A. niger* is a suitable test organism for assessing fungicidal activity. Fungi, including molds like Aspergillus, can cause various infections and spoilage. *A. niger* produces conidia (asexual spores), which can exhibit some resistance to disinfectants, although generally less so than bacterial endospores. Its inclusion helps to determine the disinfectant's effectiveness against fungal contaminants. [10,11]

Candida albicans

Candida albicans is a significant human fungal pathogen, frequently implicated in healthcare-associated infections. It is a yeast, representing a different fungal morphology compared to molds like *A. niger. C. albicans* is generally more susceptible to many disinfectants than bacterial spores or some viruses, but testing against it is essential for confirming efficacy against clinically relevant yeasts. [12]

Kocuria rosea

K. rosea is a Gram-positive bacterium that can be found in the environment and on human skin. It serves as a representative of vegetative bacteria, which are generally more susceptible to disinfectants than spores. Testing against vegetative bacteria like *K. rosea* is fundamental for demonstrating basic bactericidal activity, a primary requirement for most disinfectants. [13] The inclusion of different microbes could provide information on potential specific differences in susceptibility.

By including these microorganisms, the disinfectant study gains valuable insights into the breadth of the disinfectant's activity across different microbial kingdoms (bacteria and fungi) and forms (spores and vegetative cells), taking into consideration that this study is a series of complementary investigations and experiments covering different microorganisms in previous and forthcoming research. This comprehensive panel allows for a more thorough evaluation of the disinfectant's potential effectiveness against a range of contaminants encountered in various settings. The primary objective of this study was to determine and compare the disinfectant efficacy, quantified by LR values, of selected antimicrobial agents against four distinct microbial species. To achieve this, LR data were analyzed using robust statistical methods, including the Friedman and two-way omnibus tests, to identify significant differences in microbial susceptibility. The findings from this analysis aimed to offer practical insights for the enhancement and optimization of existing disinfection protocols. The results provide actionable insights for optimizing disinfection strategies.

MATERIALS AND METHODS

Study design and setting

Experimental design involved a dry surface contact test with an exposure time of 5 minutes under normal working conditions in the healthcare settings. Preliminary neutralization efficacy and toxicity studies have been conducted and verified elsewhere in previous laboratory studies. [14–18] The experimental design for evaluating disinfectant efficacy on dry surfaces using coupon samples was structured in several steps. The experiment framework involves different disinfectants to ensure that they are following an antimicrobial rotation program in the sanitization protocol for facilities.

Study road map and steps: Microbial groups and disinfectant efficacy testing

Appropriate sterility controls were implemented throughout the study to ensure the absence of extraneous microbial contamination. This included sterility testing of media, diluents, and coupons before use. Six microbial groups were selected to represent diverse pathogen types and resistance profiles. [7,19] Two microorganisms were tested in their sporulated forms against a sporicidal agent, while the other two were vegetative and exposed to non-sporicidal antimicrobial, that is, alcohols: [7,19,20]

- ***Aspergillus niger* (fungal spores)**: A robust fungal spore model for assessing fungicidal activity of peracetic acid-hydrogen peroxide (PA+HP) on the fungal spores.
- ***Bacillus subtilis* (bacterial spores)**: A spore-forming bacterium to evaluate the sporicidal efficacy of PA+HP.
- ***Kocuria rosea* IPA** and ***Candida albicans* IPA**: Bacterial cocci and yeast strains treated with 70% IPA to compare alcohol-based disinfectant efficacy.
- ***Kocuria rosea* ET** and ***Candida albicans* ET**: Bacterial cocci and yeast strains treated with 70% ET to compare alcohol-based disinfectant efficacy.

Microbial suspensions were prepared by serial dilution to achieve the target titer range as colony-forming units (CFU)/mL, following protocols adapted from USP <1072> guidelines.

[21,22] Ten replicates using different coupons per group were analyzed across different surface materials commonly used in healthcare and pharmaceutical facilities (denoted by two-letter codes: PG, TF, SL, RB, SS, PD, CP, EW, CS, GS). [20] Disinfectant efficacy was quantified using LR values derived from control and treatment groups via plate enumeration. [4,7] The disinfectants evaluated were: ET (70% v/v), IPA (70% v/v), and a PA+HP blend. The PA+HP blend was prepared to achieve final concentrations of 0.097% ± 0.007% PA and 0.535% ± 0.025% HP. All disinfectant solutions were prepared according to the manufacturer's instructions (if applicable) or standard laboratory procedures to ensure optimal activity and stored under normal conditions before use in closed containers.

Statistical analysis and methods

Descriptive statistics

Mean, median, standard deviation, and 95% confidence intervals were calculated. [23,24]

Outlier detection

Aberrant values (at Q = 1%) are shown with the support of a box-and-whisker diagram. [25,26]

Friedman test

Non-parametric comparison of median LR ranks across groups. [27]

Dunn's multiple comparisons

Post-hoc analysis with Benjamini-Hochberg correction ($\alpha = 0.05$).

Two-way omnibus test

Assessed the effects of microbial-disinfectant group (column factor) and surface variability (row factor). [28] Given the potential for non-normal distributions and the presence of outliers in microbiological data, the non-parametric Friedman test was selected to compare median LR ranks across the different microbial-disinfectant groups, as it does not assume normality. Dunn's multiple comparisons test with Benjamini-Hochberg correction was used as a post-hoc analysis to identify specific pairwise differences while controlling the false discovery rate. A two-way omnibus test (e.g., ANOVA, if assumptions met for transformed data, or a robust equivalent) was employed to assess the main effects of the microbial-disinfectant group (column factor) and surface variability (row factor) on LR values, and to explore potential interactions if the design allowed. The significance level (α) was set at 0.05 for all tests.

Outlier detection was performed using the 1% quartile (Q = 1%) method and supported by visual inspection of box-and-whisker diagrams. For the primary non-parametric analyses (Friedman test), identified outliers were retained in the dataset to avoid potential bias and to reflect the inherent variability often encountered in microbial responses to disinfectants. This approach acknowledges that extreme values can be biologically meaningful in disinfectant efficacy studies. The statistical analysis involved several methods to evaluate the differences in microbial count reduction:

Descriptive statistics

Measures such as mean, standard error, median, mode, standard deviation, sample variance, kurtosis, skewness, range, minimum, maximum, sum, count, and confidence level (95%) were calculated to describe the distribution of the data.

Two-way omnibus test

A two-dimensional factor examination was performed to analyze the effects of two factors (likely disinfectants and surfaces) on the microbial count reduction. The significance level (α) was set at 0.05.

Friedman test

The Friedman test, a non-parametric test, was used to assess differences in microbial count reduction across the different microorganisms.

Dunn's multiple comparisons test

Dunn's test was used as a post-hoc test following the Friedman test to perform pairwise comparisons between the microorganisms. [29–31] The α was 0.05, and the number of comparisons per family was 15. All analyses were performed using GraphPad Prism version 10 and Minitab version 17.

Ethical approval

As the research exclusively involved the use of established, non-clinical strains of microorganisms in a controlled laboratory environment and did not include human participants, human biological materials, human data, or animal subjects, the primary ethical considerations centered on responsible scientific conduct. All experimental procedures were conducted in strict adherence to institutional biosafety guidelines and relevant national/international regulations to ensure the safety of researchers and prevent any environmental release of microorganisms.

RESULTS

Descriptive statistics of antimicrobial activity

The descriptive statistics for each microorganism are presented in **Table 1** and visually summarized in **Figure 1**. This bar chart provides a visual overview of key statistical measures, including mean, median, standard deviation, sample variance, range, skewness, kurtosis, count, smallest, largest, sum, and 95% confidence level for six different microorganisms: *C. albicans* ET, *K. rosea* ET, *C. albicans* IPA, *K. rosea* IPA, *B. subtilis*, and *A. niger*.

As shown in **Table 1**, the mean LR for *B. subtilis* was 6.70, which was the highest among all microorganisms. Conversely, *A. niger* exhibited the lowest mean reduction, at 3.76. Measures of variability indicated higher standard deviation and variance for *K. rosea* IPA and *K. rosea* ET. Notably, *B. subtilis* showed zero variability in its reduction, with no recovery from surfaces. Most distributions were found to be platykurtic and negatively skewed.

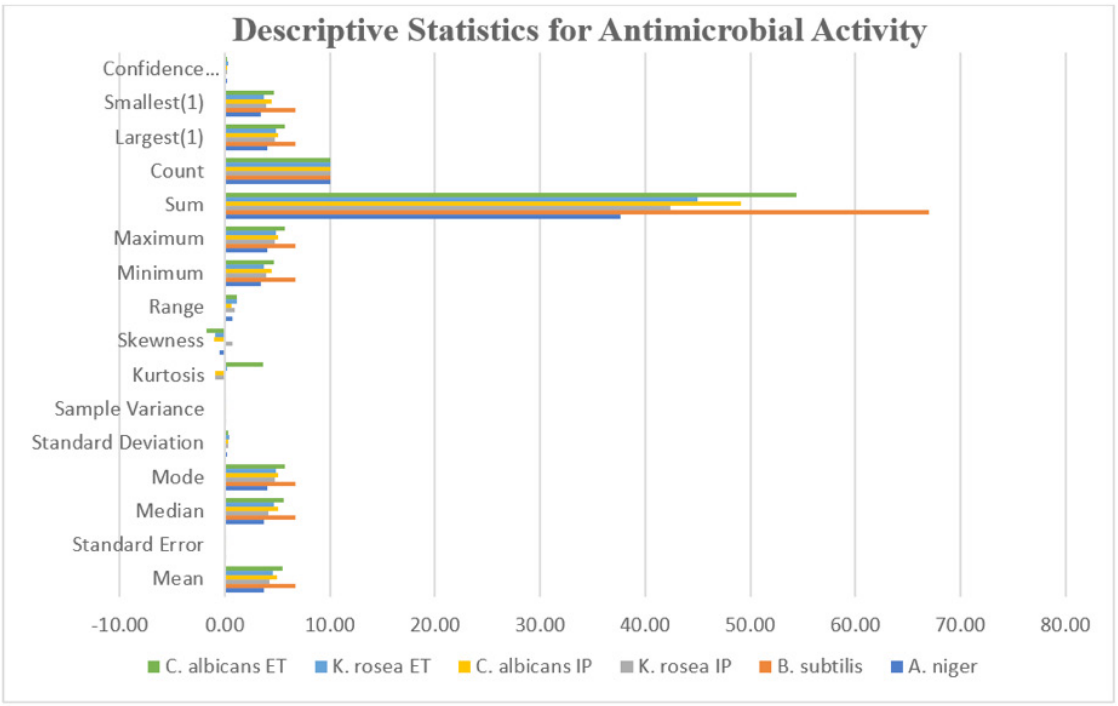
The graphical representation in **Figure 1** further highlights these observations. *B. subtilis* consistently shows the highest mean and median antimicrobial activity (around 6.70),

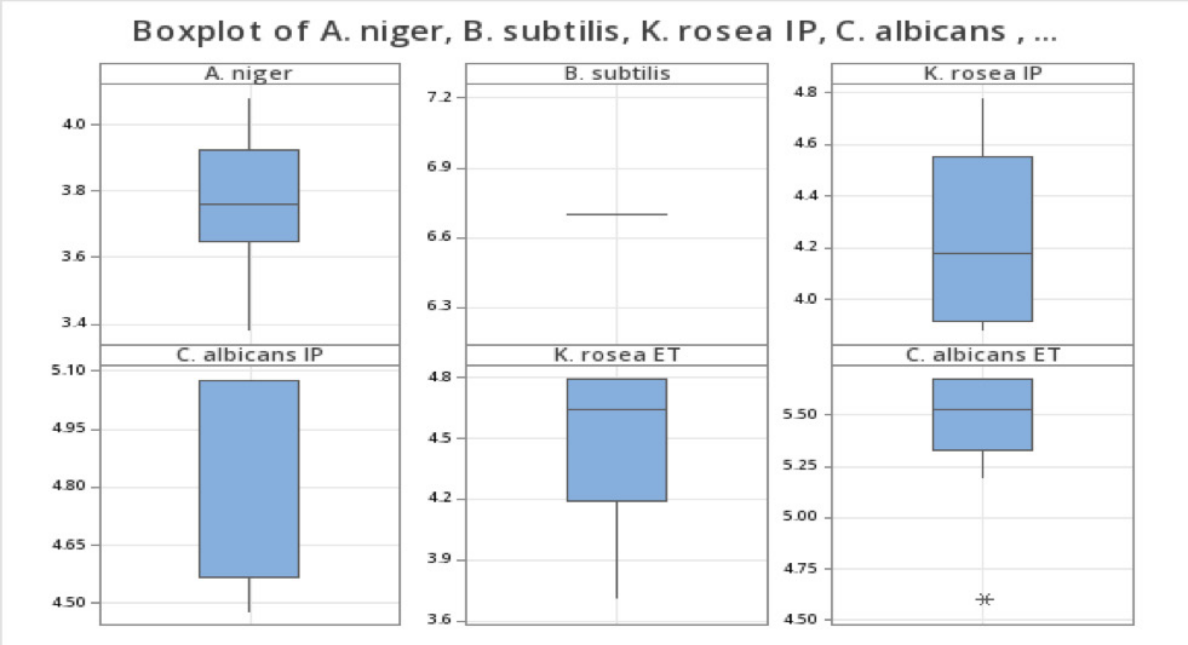
Table 1: Descriptive statistics and outlier analysis for mean logarithmic reduction values.

Microorganism	Mean ± SD	95% confidence interval	Median	Range
A. niger	3.77 ± 0.21	3.61–3.92	3.76	3.38–4.08
B. subtilis	6.70 ± 0.00	6.70–6.70	6.70	6.70–6.70
K. rosea IPA	4.24 ± 0.34	3.99–4.49	4.18	3.88–4.78
C. albicans IPA*	4.91 ± 0.27	4.72–5.11	5.08	4.48–5.08
K. rosea ET	4.49 ± 0.38	4.23–4.76	4.65	3.72–4.80
C. albicans ET	5.43 ± 0.34	5.19–5.68	5.53	4.60–5.68

IPA: isopropyl alcohol; ET: ethanol.

***Outliers**: Three outliers identified in *C. albicans* IPA (*Q* = 1%). Non-parametric tests retained outliers to avoid bias. [7]





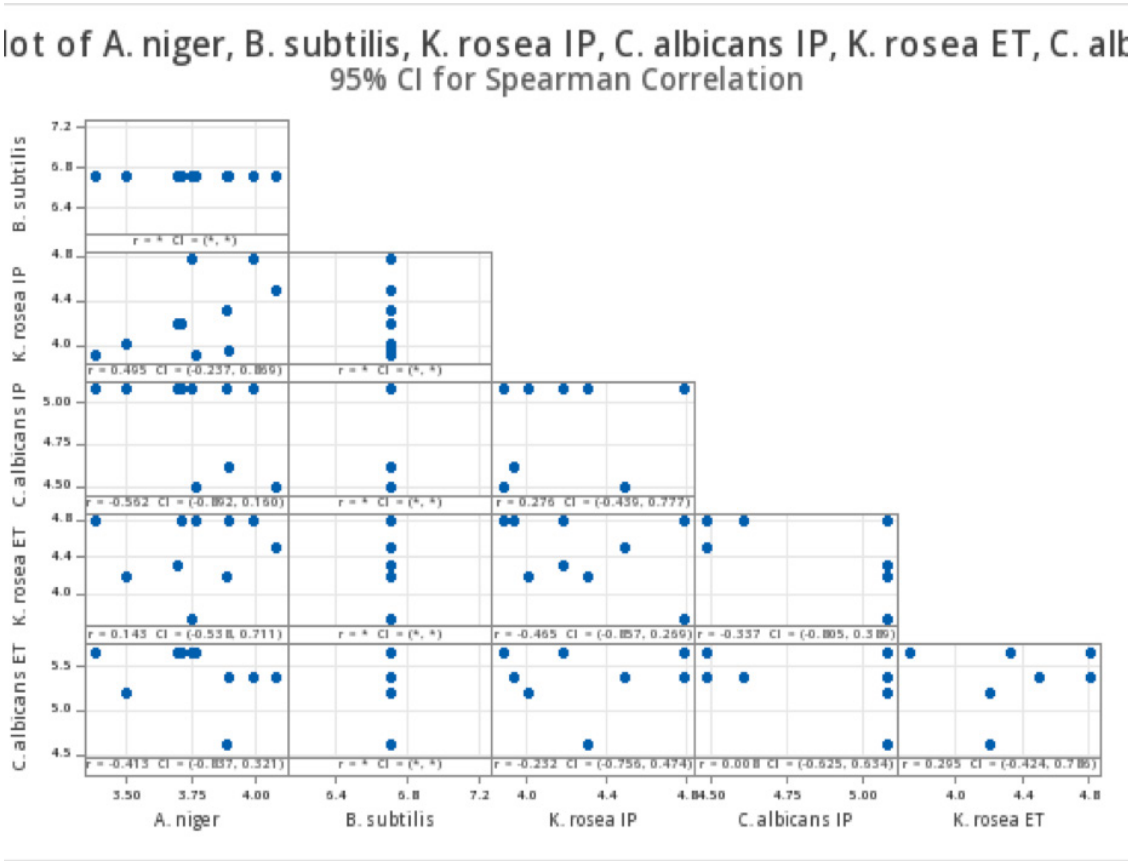


Figure 1: Descriptive statistics, boxplot, and correlation of *A. niger*, *B. subtilis*, *K. rosea* IPA, *C. albicans* IPA, *K. rosea* ET, and *C. albicans* ET.

indicating the greatest reduction. In contrast, *A. niger* shows among the lowest mean and median values (around 3.76). The other microorganisms (*C. albicans* ET, *K. rosea* ET, *C. albicans* IPA, and *K. rosea* IPA) display intermediate mean and median values. For *B. subtilis*, the remarkably low (0) standard deviation and sample variance are evident, confirming consistent high antimicrobial activity. *K. rosea* IPA and *K. rosea* ET show comparatively higher standard deviations and variances. The count bar for all six microorganisms extends to 10, confirming 10 data points per microorganism. For *B. subtilis*, the minimum and maximum values are identical to the mean. The 95% confidence level bars appear relatively similar across all microorganisms.

Microbial recovery and disinfectant efficacy

Figure 2 displays the average number of microbial CFU, scaled by 105, recovered from surface samples for each microorganism under various conditions, plotted on a logarithmic scale. The *y*-axis represents the mean recovered count, ranging from 10^{-6} to 10^{2}. The *x*-axis categorizes data by microorganism and condition (P+, Ts, PA+HP, IPA: 70%, ET: 70%).

Table 2 provides detailed LR data. The "P+" columns indicate the initial microbial load (log_{10} units), while the "Ts" columns and values under specific disinfectant-microorganism combinations represent the LR after a 5-minute exposure.

Table 2 shows that *B. subtilis* consistently achieved a high LR of 6.70 when treated with PA+HP. However, *B. subtilis* showed a consistent 0.00 LR with IPA 70% and ET 70%. *C. albicans* IPA also exhibited a consistent 0.00 LR under IPA 70% and ET 70%. *A. niger* demonstrated a substantial LR with PA+HP (5.94) but generally lower and more variable LRs with IPA 70% (ranging from 1.87 to 2.57). *K. rosea* and *C. albicans* ET strains displayed variable LRs across disinfectants and surfaces.

Inferential statistical analysis

Descriptive statistics of antimicrobial activity

Descriptive statistics for each microorganism are presented in **Table 1** and **Figure 1**. *Bacillus subtilis* showed the highest mean LR of 6.70, while *Aspergillus niger* exhibited the lowest mean reduction at 3.76. Measures of variability, including standard deviation and variance, were higher for *Kocuria rosea* IPA and *Kocuria rosea* ET. *B. subtilis* demonstrated 0 variability in its reduction, with no recovery from surfaces. Most distributions were platykurtic and negatively skewed. The mean and median antimicrobial activity for *B. subtilis* was consistently high (around 6.70), indicating the greatest reduction. In contrast, *A. niger* showed the lowest mean and median values (around 3.76). Other microorganisms (*C. albicans* ET, *K. rosea* ET, *C. albicans* IPA, and *K. rosea* IPA) displayed intermediate mean and median values. For *B. subtilis*, the minimum and maximum values were identical to the mean. Ten replicates were analyzed for all six microorganisms.

The Spearman correlation analysis (**Figure 1**; 95% CI for Spearman correlation) examined the relationships between pairs of microorganisms in LR values across the tested surfaces.

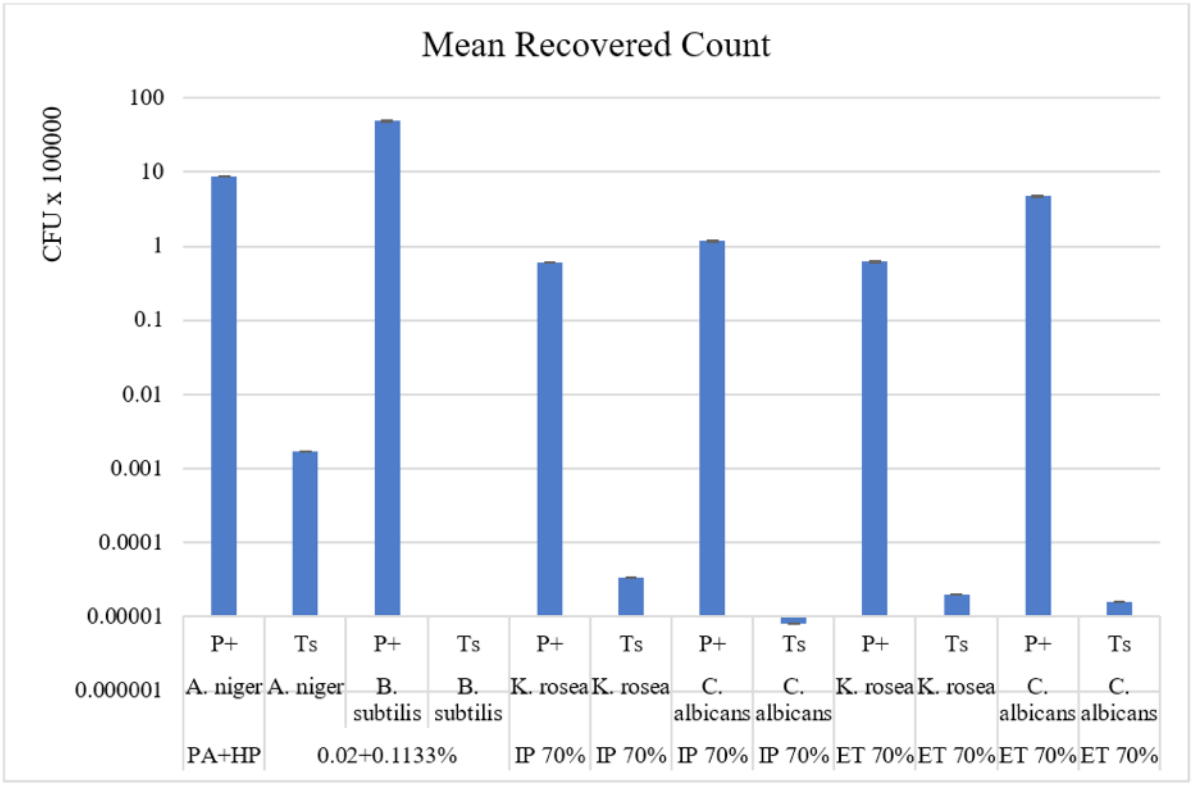


Figure 2: Absolute microbial recovery count from surface samples for each microorganism-disinfectant combination.

Table 2: Disinfectant efficacy test for selected microorganisms on different surface samples.

Disinfectant	PA+HP 0.097+0.54%*				IPA 70%				ET 70%			
M.O.	*A. niger*		*B. subtilis*		*K. rosea*		*C. albicans*		*K. rosea*		*C. albicans*	
Surface	P+	Ts	P+	Ts	P+	Ts	P+	Ts	P+	Ts	P+	Ts
PG	5.94	2.20	6.70	0.00	4.78	0.00	5.08	0.00	4.80	1.08	5.68	0.00
TF		2.23		0.00		0.60		0.00		0.00		0.00
SL		1.96		0.00		0.00		0.00		0.00		0.30
RB		2.24		0.00		0.60		0.00		0.48		0.00
SS		2.05		0.00		0.48		0.00		0.60		1.08
PD		2.45		0.00		0.78		0.00		0.60		0.48
CP		2.57		0.00		0.90		0.00		0.00		0.00
EW		2.05		0.00		0.85		0.48		0.00		0.30
CS		1.87		0.00		0.30		0.60		0.30		0.30
GS		2.18		0.00		0.90		0.60		0.00		0.00

IPA: isopropyl alcohol; ET: ethanol.

*Peracetic acid (PA): 0.0009–0.0010 (or 0.09%–0.10%) and hydrogen peroxide (HP): 0.0051–0.0056 (or 0.51%–0.56%).

Most correlations observed were weak to moderate. For example, the highest absolute value was observed between *A. niger* and *C. albicans* (IPA), with a negative correlation of $r = -0.56$, followed by *K. rosea* (IPA) with a positive correlation of $r = 0.495$. While the lowest was between the *C. albicans* ET group versus the IPA group, with $r = 0.00$

Microbial recovery and disinfectant efficacy

Mean recovered microbial CFU from surface samples for each microorganism under various conditions are shown in **Figure 2**. Detailed LR data are provided in **Table 2**. *B. subtilis* consistently achieved a high LR of 6.70 when treated with the PA+HP blend. However, *B. subtilis* showed a consistent 0.00 LR with IPA 70% and ET 70%. *C. albicans* IPA also exhibited a consistent 0.00 LR under IPA 70% and ET 70%. *A. niger* demonstrated a substantial LR with PA+HP (5.94) but generally lower and more variable LRs with IPA 70% (ranging from 1.87 to 2.57). *K. rosea* and *C. albicans* ET strains displayed variable LRs across disinfectants and surfaces.

Inferential statistical analysis

The results of the Friedman test and Dunn's post-hoc comparisons are presented in **Table 3**. The Friedman test indicated overall significance (χ^2 = 45.40; p < 0.0001), suggesting significant differences in microbial count reduction across the different microorganisms and disinfectant treatments. Dunn's post-hoc comparisons identified specific significant differences: *B. subtilis* versus *A. niger* (rank sum difference: 49.00; p < 0.0001); *C. albicans* ET versus *A. niger* (rank sum difference: 38.00; p < 0.0001); *K. rosea* IPA versus *B. subtilis* (rank sum difference: −37.50; p = 0.0001); *C. albicans* IPA versus *A. niger* (rank sum difference: 26.50; p = 0.0232); *C. albicans* ET versus *K. rosea* IPA (rank sum difference: 26.50; p = 0.0232); and *K. rosea* ET versus *B. subtilis* (rank sum difference: −30.00; p = 0.0051).

- **Column factor (microbe-disinfectant group)**: Explained 92.36% of variance (F = 123.9; p < 0.0001).
- **Row factor (surface variability)**: Negligible impact (0.93%; F = 0.695; p = 0.7097).
- **Interaction**: Assumed absent due to no replicates per cell.

The two-way omnibus test results are also summarized in **Table 3**. The column factor (microbe-disinfectant group) explained 92.36% of the variance (F = 123.9; p < 0.0001). The row factor (surface variability) showed a negligible impact (0.93% variance explained; F = 0.695; p = 0.7097). Interaction was assumed absent due to the lack of replicates per cell. The parametric test results were nearly identical to the non-parametric equivalent.

DISCUSSION

This study evaluated the efficacy of different disinfectants, including PA+HP, 70% IPA, and 70% ET, against six diverse microbial groups on ten different dry surface materials commonly found in healthcare and pharmaceutical facilities. The findings provide crucial insights into disinfectant performance, supporting the implementation of effective antimicrobial rotation programs. The descriptive statistics (**Table 1**; **Figure 1**) and inferential statistical tests (**Table 3**) consistently highlight significant differences in disinfectant efficacy across the various microorganisms. *B. subtilis* demonstrated the highest mean LR (6.70), indicating it was the most susceptible microorganism to the tested sporicidal product, PA+HP. [32] This near-complete and uniform killing of *B. subtilis* (reflected by its 0 variability, and consistent with previous data) suggests the strong sporicidal action of PA+HP. This maximal efficacy against *B. subtilis* is likely attributable to the specific sporicidal mechanism of PA+HP.

Conversely, *A. niger* exhibited the lowest mean reduction (3.76), indicating its higher resistance or lower susceptibility to the sporicidal product at the tested exposure time. [32,33] This resistance of *A. niger* aligns with the known protective properties of its melanin-rich fungal spores, which can act as a barrier to disinfectant penetration. The data further revealed that *B. subtilis* consistently showed a 0.00 LR when exposed to IPA 70% and ET 70%. This stark contrast to PA+HP efficacy underscores its high resistance to alcohol-based disinfectants, which is expected given its spore-forming capability; alcohols are generally not effective against bacterial endospores. Similarly, *C. albicans* IPA consistently showed a almost nil recovery with IPA 70% and ET 70%, which was an expected finding and confirms that alcohols are highly effective against this vegetative yeast strain, resulting in complete inactivation with no microbial recovery under the test conditions.

The results also indicate that *A. niger* showed substantial LR with PA+HP (5.94), confirming its susceptibility to this sporicidal agent, but generally lower and more variable LRs with IPA 70% (ranging from 1.87 to 2.57). This suggests differential efficacy depending on the disinfectant used and potential variability in response across surfaces. The *K. rosea* and *C. albicans* ET strains exhibited variable LRs across the disinfectants and surfaces, indicating differing levels of susceptibility. The variability measures (standard deviation,

Table 3: Friedman test and Dunn's post-hoc comparisons with overall significance: χ^2 = 45.40, p < 0.0001 (approximate p due to tied ranks) and pairwise comparisons (adjusted p values).

Comparison†	Rank sum difference	Significance	Adjusted p
B. subtilis vs. *A. niger*	49.00	****	<0.0001
C. albicans ET vs. *A. niger*	38.00	****	<0.0001
K. rosea IPA vs. *B. subtilis*	−37.50	***	0.0001
C. albicans IPA vs. *A. niger*	26.50	*	0.0232
C. albicans ET vs. *K. rosea* IPA	26.50	*	0.0232
K. rosea ET vs. *B. subtilis*	−30.00	**	0.0051

IPA: isopropyl alcohol; ET: ethanol.

*P < 0.1; **P < 0.01; ***P < 0.001;****P < 0.0001.

† Two-way omnibus:

sample variance, and range in **Figure 1** and **Table 1**) are particularly crucial. The higher standard deviations and variances observed for *K. rosea* IPA and *K. rosea* ET suggest less consistent disinfectant efficacy for these species across the tested conditions (including surfaces and disinfectant types). [33,34] This highlights that while disinfectants may be effective on average, their performance can be inconsistent for certain microorganisms, which is vital for real-world applications.

Skewness and Kurtosis (**Figure 1**) provide further insights into the distribution shapes of the antimicrobial activity measurements. The variations observed indicate that the data distributions are not uniformly normal, supporting the appropriateness of using non-parametric tests like the Friedman test. [34,35] Hence, non-parametric methods are crucial for analyzing microbiological data, especially when the data contain outliers or exhibit skewed distributions.

The inferential statistical analysis (**Table 3**) strongly supports these observations. The highly significant column factor (microbial-disinfectant group) in the two-way omnibus test ($p < 0.0001$) directly reflects the prominent differences in LR patterns among the microorganisms presented in **Table 2**. The consistent high efficacy against *B. subtilis* with PA+HP, coupled with *A. niger*'s high tolerance, and the varied responses of other microorganisms to different disinfectants, are the primary drivers of these significant overall effects. [1,36,37] Dunn's post-hoc comparisons further pinpoint these differences, for instance, by showing highly significant distinctions between *B. subtilis* versus *A. niger* and *B. subtilis* versus *K. rosea* species, which directly stem from the drastically different LR values observed (e.g., complete vs. zero reduction with alcohol-based disinfectants).

Conversely, the non-significant row factor (surface variability) in the two-way omnibus test ($p = 0.7097$) suggests that, on average, the type of surface material did not independently cause significant variations in the overall LR values across all microorganisms and disinfectants tested. [33–36,38–42] This implies that while surface effects might still be present for specific microorganism-disinfectant combinations, they did not exert a broad, overarching influence on disinfectant efficacy in this study.

Figure 2, which visualizes the absolute microbial recovery counts, complements the LR data by showing the number of surviving microorganisms, offering a direct perspective on the practical outcome of disinfection in terms of actual microbial survival. [1,36,38] This representation reinforces the statistical findings by visually illustrating the substantial differences in mean recovered counts between the microorganisms under disinfectant challenge, confirming the varying levels of reduction achieved.

The Spearman correlation analysis, presented below the boxplot in **Figure 1**, provides additional context by indicating the extent to which microorganisms tend to respond similarly or differently to the disinfectant across varying conditions. Finding weak to moderate correlations implies that factors influencing disinfectant effectiveness against one microorganism are only partially related to those influencing it against another. [1,43,44] This suggests that while there may be some shared environmental influences from the surface type or disinfectant application, the microorganisms' responses are also influenced by their intrinsic biological differences and potentially unique interactions with the disinfectant or surface. This underscores the complexity of disinfectant efficacy, which can vary significantly depending on the specific microorganism, even under the same environmental conditions. [43,45] The presence of these correlations, even if weak, indicates some level of shared influence or interconnectedness in susceptibility patterns on different surfaces when exposed to disinfectants. [43–45]

Beyond the general trends, specific key findings emerged from our analysis, offering critical insights into disinfectant performance. [1,46,47] First, we observed maximal efficacy against *B. subtilis*, which achieved near-sterilization with an LR of 6.70. This high level of effectiveness is likely attributable to the potent sporicidal action of the PA+HP disinfectant. [37] Conversely, minimal efficacy was noted for *A. niger*, which exhibited considerable resistance with an LR of only 3.77. This finding aligns with the understanding that *A. niger*'s melanin-rich spores can effectively block disinfectant penetration, contributing to its resilience. [46] Lastly, in an alcohol comparison, ET demonstrated superior performance over IPA against *C. albicans*. ET yielded an LR of 5.43 compared to IPA's 4.91, with a statistically significant difference ($p = 0.0232$). This enhanced efficacy of ET is likely due to its more effective membrane disruption capabilities against this yeast.

The study found that 70% ET was statistically more effective against *C. albicans* than 70% IPA ($p = 0.0232$). Practically, ET achieved a 0.52 higher mean LR against *C. albicans* (LR = 5.43 ± 0.34 for ET vs. LR = 4.91 ± 0.27 for isopropanol), indicating a substantially greater reduction in viable yeast cells. This approach is amplified when applied to other key comparisons where practical significance is important. [47] For instance, when comparing *B. subtilis* to *A. niger*, *B. subtilis* (LR = 6.70) showed a nearly 3-log greater reduction with PA+HP compared to *A. niger* (LR = 3.77), highlighting a vastly different practical outcome in sporicidal efficacy.

This study has a few limitations. First, the experiments were conducted under specific laboratory conditions (e.g., 5-minute exposure time, specific surface types), and results may vary under different real-world environmental conditions or with different contact times or concentrations. [Second, while ten common surface materials were used, the study did not explore an exhaustive list of all materials found in healthcare or pharmaceutical settings. Third, the study focused on a specific set of four microorganisms; the findings may not be generalized to all types of bacteria, fungi, or viruses. [48–58] Additionally, the mechanisms of resistance or action were inferred rather than directly investigated at a molecular level. Future research should address these limitations.

Nevertheless, this comprehensive analysis provides a clear and detailed picture of the differential susceptibility and response variability of the tested microorganisms to disinfectants on dry surfaces. The findings are fundamental to understanding and improving disinfection protocols in healthcare settings, emphasizing that disinfectant choices should consider the specific microbial threats to ensure optimal efficacy within an antimicrobial rotation program. Future studies should explore molecular mechanisms of resistance and optimize disinfectant blends for broad-spectrum activity. In addition,

an antimicrobial rotation program should be considered for microbial level control in healthcare and pharmaceutical settings to mitigate the risk of microbial endurance of the applied antimicrobials.

CONCLUSION

Effective sanitization could be demonstrated in the study during the postulated exposure time frame. However, based on the targeted area and activity, the criticality might necessitate extending exposure time, changing the concentration, or even the type of disinfectants to achieve effective disinfection. The statistical analysis demonstrated a significant reduction in microbial counts across all microorganisms. However, the extent of this reduction varied significantly between species. *B. subtilis* exhibited the highest susceptibility to the sporicidal disinfectant treatment, while *A. niger* showed the lowest. The type of microorganism significantly influenced the reduction of microbial counts, whereas the surface type did not have a significant effect. These findings suggest that the efficacy of the disinfectant is more dependent on the microbial species than the surface it is applied to. Further research is warranted to explore the specific factors contributing to the differential susceptibility of these microorganisms, including their intrinsic properties and interactions with the disinfectant. This study demonstrates that microbial morphology and disinfectant chemistry critically determine decontamination efficacy. Moreover, this study highlighted several concerning issues. Sporicidal agents essential: *B. subtilis*'s near-complete eradication underscores the need for sporicides (e.g., PA+HP) in high-risk settings. Fungal spore challenges: *A. niger*'s resilience necessitates prolonged contact times or synergistic formulations. Alcohol Selection: ET is preferable for *C. albicans*, but IPA may suffice for less resistant strains. Methodological Insights: Non-parametric methods are vital for microbiological data with outliers and skewed distributions. Nevertheless, a limited approach to parametric tests could be investigated with caution and by case-by-case selection.

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CONFLICT OF INTEREST

None.

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