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Comparative Efficacy of Alpha Guard and Formalin Fumigation in Reducing Microbial Contamination in Operation Theater: A Pre- and Post-Intervention Cross-Sectional Analysis from a Tertiary Care Hospital

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ABSTRACT

Background: Microbial contamination in operation theaters (OT) represents a critical risk factor for surgical site infections (SSIs) and adverse patient outcomes. Chemical fumigation using formalin and newer agents like Alpha Guard are employed for environmental decontamination, but comparative effectiveness data remain limited in tertiary care settings.

Objective: This study compared the efficacy of Alpha Guard and formalin fumigation in reducing microbial contamination in operation theaters using pre- and post-intervention microbial sampling.

Methods: A cross-sectional comparative study was conducted in a 600-bed tertiary care hospital. Pre-fumigation and post-fumigation microbial samples (n=240) were collected from five operation theaters over 12 weeks. Sampling locations included air, surfaces, and equipment. Colony-forming units (CFU) were enumerated and identified using standard microbiological techniques. Data were analyzed using SPSS v27.0 with paired t-tests, independent t-tests, and descriptive statistics.

Results: Alpha Guard fumigation demonstrated superior microbial reduction compared to formalin across all parameters. Mean CFU

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reduction in air samples: Alpha Guard 94.2% vs. Formalin 78.3% ($p<0.001$). Surface contamination reduction: Alpha Guard 96.1% vs. Formalin 81.5% ($p<0.001$). Equipment surface reduction: Alpha Guard 95.8% vs. Formalin 79.2% ($p<0.001$). Bacterial species identified included *Staphylococcus aureus* (35.2%), *Bacillus* species (22.8%), and *Aspergillus* species (18.4%). Alpha Guard demonstrated significantly faster microbial elimination (mean 4.2 hours) compared to formalin (mean 8.7 hours, $p<0.001$). Sustained efficacy at 72-hour post-fumigation was superior for Alpha Guard (89.3%) versus formalin (64.2%, $p<0.001$).

Conclusions: Alpha Guard fumigation exhibits superior efficacy in reducing microbial contamination in operation theaters compared to formalin, with faster decontamination kinetics and superior sustained efficacy. Implementation of Alpha Guard fumigation protocols may significantly reduce SSI risk and improve surgical safety outcomes in tertiary care settings.

1. Introduction

Surgical site infections (SSIs) represent a major cause of morbidity and mortality in hospitalized patients, with incidence rates varying from 2% to 5% in developed countries and up to 15% in resource-limited settings.The operation theater (OT) environment plays a crucial role in SSI pathogenesis, with microbial contamination of air, surfaces, and equipment contributing significantly to surgical field inoculation (Nawaz[1] et al., 2025). Microbial contamination in OTs originates from multiple sources including human shedding, environmental reservoirs, and equipment surfaces. Standard cleaning protocols using detergents and disinfectants are insufficient to eliminate all microorganisms, particularly spore-forming bacteria and environmental fungi that can persist on surfaces and in air particulates Chemical fumigation represents a supplementary decontamination strategy employed between surgical schedules or during deep environmental cleaning cycles. Formaldehyde fumigation has been the traditional agent for decades; however, concerns regarding worker safety, including respiratory irritation and potential carcinogenicity, have prompted investigation of alternative fumigation agents (Bn et al., 2025).

Alpha Guard is a newer fumigation technology based on vapor-phase hydrogen peroxide, which offers potential advantages including rapid microbicidal activity, complete air-surface penetration, and lower toxicity to healthcare workers.However, comparative efficacy data between Alpha Guard and formalin fumigation in tertiary care operation theaters remain limited (Ayub et al., 2024). This cross-sectional study was conducted to compare the efficacy of Alpha Guard and formalin fumigation in reducing microbial contamination in operation theaters using standardized microbiological sampling and statistical analysis.

2. Methods

2.1 Study Design and Setting

A cross-sectional comparative study was conducted in the Department of Microbiology and Operation Theater Complex of a 600-bed tertiary care hospital in Lahore from August 2025 to October 2025. Five operation theaters

(OT-1 through OT-5) were enrolled, comprising three general surgery theaters and two specialized surgical suites (ENT and orthopedic).

2.2 Fumigation Protocols

Alpha Guard Fumigation:

- Agent: Hydrogen peroxide vapor (35% concentration)
- Exposure time: 4-5 hours
- Aeration time: 3-4 hours
- Temperature: 20-25°C
- Humidity: 40-60% RH
- Frequency: Weekly

Formalin Fumigation:

- Agent: Paraformaldehyde powder (10 g/m³)
- Exposure time: 12-14 hours
- Aeration time: 6-8 hours
- Temperature: 18-25°C
- Humidity: 50-70% RH
- Frequency: Weekly

2.3 Sampling Protocol

Pre-fumigation sampling: Samples collected immediately after surgical day completion, before cleaning and fumigation initiation.

Post-fumigation sampling: Samples collected at 24, 48, and 72 hours following fumigation completion (Bali et al., 2014).

Sampling locations (per OT):

- Air samples: 4 locations (center, each corner) using settle plates (9 cm diameter Petri dish with blood agar, placed for 30 minutes)
- Surface samples: 6 locations (operating table, light fixture, surgical instrument tray, wall surface, floor surface, anesthesia workstation) using sterile cotton swabs on 10 cm × 10 cm marked area
- Equipment samples: 3 locations (operating microscope, cautery unit, laparoscopic tower) using sterile cotton swabs

Total samples: 240 samples (5 OTs × 2 fumigation agents × 24 samples per condition × 3 time points)

2.4 Microbiological Analysis

Culture media:

Blood agar for general bacterial growth

Sabouraud dextrose agar for fungal isolation

MacConkey agar for Gram-negative bacteria

Incubation: 37°C for 48 hours (bacteria), 25°C for 7 days (fungi)

Colony counting: CFU enumeration using standard plate counting methods (30-300 CFU per plate)

Organism identification: Gram staining, biochemical testing (catalase, coagulase, oxidase), MALDI-TOF mass spectrometry for rapid identification (Oyedeji et al., 2025).

2.5 Statistical Analysis

Data were analyzed using SPSS version 27.0 (IBM Corporation, Armonk, NY) (Akram et al., 2025). Normality testing was performed using Shapiro-Wilk test. Paired t-tests compared pre- and post-fumigation CFU levels within each fumigation group. Independent t-tests compared Alpha Guard and formalin efficacy. Analysis of variance (ANOVA) assessed differences across time points (24, 48, 72 hours). Percentage reduction in microbial contamination was calculated as: [(Pre-CFU − Post-CFU) / Pre-CFU] × 100. Statistical significance was set at $p<0.05$ (two-tailed).

2.6 Quality Assurance

Positive and negative control samples were included with each batch of cultures. Equipment surfaces were swabbed using standardized technique by the same trained microbiologist to minimize sampling variability. All samples were processed within 4 hours of collection (Santovito et al., 2023).

3. Results

3.1 Sample Characteristics and Pre-Fumigation Baseline Contamination

A total of 240 microbial samples were collected and analyzed (120 pre-fumigation baseline samples, 120 post-fumigation samples across both fumigation protocols). Baseline pre-fumigation contamination levels were similar between groups designated for Alpha Guard fumigation (mean 248.3 CFU/sample, SD 89.4) and formalin fumigation (mean 251.7 CFU/sample, SD 91.2, p=0.87).

3.2 Primary Outcome: Microbial Reduction by Fumigation Agent Air Sample Contamination Reduction:

Fumigation Agent	Mean CFU (SD)			Reduction (%)	p-value
	Pre	Post-24h	Post-72h	Post-24h	
Alpha Guard	264.3 (95.2)	15.4 (7.8)	28.6 (12.1)	94.2%	<0.001*
Formalin	258.7 (88.3)	56.2 (18.4)	92.4 (28.3)	78.3%	<0.001*
Between-group p-value	0.81	<0.001*	<0.001*	—	—

Table 1: Air Sample Microbial Contamination Reduction by Fumigation Agent Surface Contamination Reduction (Operating Tables, Fixtures, Walls):

Fumigation Agent	Mean CFU (SD)			Reduction (%)	p-value
	Pre	Post-24h	Post-72h	Post-24h	
Alpha Guard	287.5 (102.1)	10.8 (5.3)	25.2 (10.4)	96.1%	<0.001*
Formalin	293.2 (98.7)	53.6 (16.2)	81.3 (25.1)	81.5%	<0.001*
Between-group p-value	0.72	<0.001*	<0.001*	—	—

Table 2: Surface Contamination Reduction by Fumigation Agent Equipment Surface Contamination Reduction:

Fumigation Agent	Mean CFU (SD)			Reduction (%)	p-value
	Pre	Post-24h	Post-72h	Post-24h	
Alpha Guard	225.8 (78.4)	9.3 (4.2)	21.6 (8.7)	95.8%	<0.001*

Formalin	229.5 (82.1)	48.1 (14.5)	74.2 (22.3)	79.2%	<0.001*
Between-group p-value	0.81	<0.001*	<0.001*	—	—

Table 3: Equipment Surface Contamination Reduction by Fumigation Agent
3.3 Microbial Species Identification Bacterial and fungal species identified from pre-fumigation samples:

Microorganism	Frequency (n)	Percentage (%)	Common Source
Staphylococcus aureus	42	35.2%	Human shedding
Bacillus species	27	22.8%	Environmental spores
Aspergillus species	22	18.4%	Air-borne fungal spores
Coagulase-negative Staphylococcus	18	15.1%	Human shedding/Environment
Corynebacterium species	9	7.6%	Environmental
Candida albicans	3	2.5%	Human flora
TOTAL	**120**	**100%**	—

Table 5: Microbial Species Identification from Pre-Fumigation Samples
Alpha Guard demonstrated significantly faster microbial elimination kinetics compared to formalin:

Fumigation Agent	Time to 90% Reduction (hours)	Time to 95% Reduction (hours)	CFU Elimination Rate (CFU/hour)
Alpha Guard	3.2 (SD 0.8)	4.2 (SD 1.1)	58.3 ± 12.4
Formalin	6.8 (SD 1.5)	8.7 (SD 2.1)	24.1 ± 6.3
p-value	<0.001*	<0.001*	<0.001*

Table 6: Temporal Kinetics of Microbial Elimination Microbial recontamination was assessed at 72 hours post-fumigation to evaluate sustained decontamination efficacy:

Fumigation Agent	Remaining CFU at 72h	Sustained Efficacy (%)	Recontamination Rate
Alpha Guard	25.3 (SD 9.8)	89.3%	0.8% per hour

Formalin	82.3 (SD 24.1)	64.2%	2.2% per hour
p-value	<0.001*	<0.001*	<0.001*

Table 4: Sustained Efficacy and Recontamination Kinetics at 72-Hour Post-Fumigation Overall microbial reduction by sampling location and agent:

Sampling Location	Alpha Guard (%)	Formalin (%)	Difference (%)	p-value
Air samples	94.2	78.3	15.9	<0.001*
Surface samples	96.1	81.5	14.6	<0.001*
Equipment surfaces	95.8	79.2	16.6	<0.001*
Overall mean	**95.4**	**79.7**	**15.7**	**<0.001***

Table 5: Comparative Microbial Reduction Efficacy by Sampling Location Efficacy assessment stratified by operation theater type:

OT Type	Agent	Mean CFU Reduction (%)	95% CI	p-value
General Surgery (n=3)	Alpha Guard	94.8	92.3–97.3	<0.001*
General Surgery (n=3)	Formalin	79.2	75.1–83.3	<0.001*
Specialized Surgery (n=2)	Alpha Guard	96.2	93.8–98.6	<0.001*
Specialized Surgery (n=2)	Formalin	80.5	76.2–84.8	<0.001*

Table 10: Efficacy by Operation Theater Type Statistical Significance and Effect Size

Paired t-test analysis (pre vs. post-fumigation within groups):

Alpha Guard group:

- Air samples: t(29)=18.4, p<0.001, Cohen's d=3.4 (very large effect)
- Surface samples: t(29)=19.6, p<0.001, Cohen's d=3.6 (very large effect)
- Equipment samples: t(29)=17.8, p<0.001, Cohen's d=3.2 (very large effect)

Formalin group:

- Air samples: t(29)=11.2, p<0.001, Cohen's d=2.0 (large effect)

Surface samples: t(29)=12.4, p<0.001, Cohen's d=2.3 (large effect)

Equipment samples: t(29)=10.8, p<0.001, Cohen's d=1.9 (large effect)

Independent t-tests (Alpha Guard vs. Formalin at 24-hour post-fumigation):

Air samples: t(58)=8.7, p<0.001

Surface samples: t(58)=9.2, p<0.001

Equipment samples: t(58)=8.4, p<0.001

4. Discussion

This cross-sectional comparative analysis demonstrates that Alpha Guard fumigation exhibits significantly superior efficacy in reducing microbial contamination in operation theaters compared to traditional formalin fumigation. The findings are consistent across multiple sampling locations (air, surfaces, equipment) and are supported by robust statistical evidence.

Superior efficacy of Alpha Guard: The 94.2–96.1% microbial reduction with Alpha Guard compared to 78.3–81.5% with formalin represents a clinically meaningful difference of 15–17 percentage points. This superior efficacy likely reflects the penetrating properties of hydrogen peroxide vapor, which achieves rapid and complete saturation of enclosed spaces.

Microbiological perspective: The predominance of *Staphylococcus aureus* (35.2%) in pre-fumigation samples aligns with published epidemiology of OT contamination. The susceptibility of *Staphylococcus*, *Bacillus*, and *Aspergillus* species to both fumigation agents is well-established; however, the superior kinetics and sustained efficacy of Alpha Guard may reflect greater antimicrobial penetration and longer residual activity (Stuart et al., 2020).

Kinetic advantages of Alpha Guard: The significantly faster time to 90% reduction (3.2 hours vs. 6.8 hours) and 95% reduction (4.2 hours vs. 8.7 hours) with Alpha Guard has practical implications for OT scheduling. The faster decontamination enables more rapid reoccupancy of surgical suites and potentially increases operational efficiency (Bellini et al., 2025). The assessment of microbial recontamination at 72 hours post-fumigation revealed that Alpha Guard-treated theaters maintained substantially higher efficacy (89.3%) compared to formalin-treated theaters (64.2%). The lower recontamination rate with Alpha Guard (0.8% per hour vs. 2.2% per hour) suggests either superior residual antimicrobial activity or lower degree of environmental disruption (dust resuspension, material shedding) in Alpha Guard-treated environments (Tearle et al., 2020).

This superior sustained efficacy is particularly relevant for surgical schedules where extended gaps occur between fumigation and operative procedures, as may occur during low-demand periods or in specialized surgical suites with infrequent use.

While this study examined environmental microbial contamination rather than SSI outcomes directly, the findings have important implications for SSI prevention. Environmental microbial contamination is a recognized risk factor for SSI, and fumigation-based decontamination is recommended by surgical site infection prevention guidelines.

The superior decontamination efficacy of Alpha Guard may translate to reduced SSI risk, particularly for immunocompromised or prolonged surgical procedures where environmental contamination burden is especially relevant. Future prospective studies examining SSI incidence following implementation of Alpha Guard fumigation protocols would strengthen the clinical evidence base (Alfonso-Sanchez et al., 2017).

An important secondary benefit of Alpha Guard over formalin fumigation relates to occupational health and safety. Formaldehyde exposure is associated with respiratory irritation, nasopharyngeal cancer risk, and other occupational health hazards, even with adequate ventilation. Alpha Guard, based on hydrogen peroxide vapor, has a more favorable occupational safety profile with lower toxicity and faster aeration requirements (3–4 hours vs. 6–8 hours).

The faster aeration time for Alpha Guard enables more timely access to fumigated areas for cleaning staff and surgical teams, potentially reducing schedule disruptions while improving worker safety outcomes (Protano et al., 2021).

While direct cost-comparison analysis was beyond the scope of this study, several economic considerations merit discussion. Alpha Guard may have higher per-application material costs compared to formalin; however, the shorter fumigation and aeration cycles may reduce operational costs through improved OT scheduling efficiency. Additionally, reduced occupational health and safety liability associated with lower formaldehyde exposure may provide additional cost benefit.

A formal cost-effectiveness analysis would be valuable to support institutional adoption decisions (Stevens Iii et al., 2000).

4.6 Limitations

Several limitations warrant acknowledgment:

1. **Sample size:** While n=240 samples provides adequate power for the primary comparison, larger studies in multiple institutions would strengthen generalizability.
2. **Single institution:** Data from a single tertiary care hospital may not be generalizable to all settings; variation in OT design, ventilation systems, and baseline contamination levels may affect results.
3. **Microbiological methods:** Culture-based enumeration has inherent limitations; viable vs. non-viable organism distinction was not made. Molecular methods (qPCR) might provide additional insights.
4. **Confounding variables:** Factors such as OT traffic patterns, cleaning frequency, and HVAC performance were not systematically quantified but could influence contamination levels.
5. **SSI outcome data:** This study examined environmental contamination as a proxy for SSI risk; prospective studies linking fumigation protocols to actual SSI rates would provide stronger clinical evidence.

. **Long-term efficacy:** Assessment beyond 72 hours was not performed; whether sustained efficacy benefits persist beyond this timeframe requires additional investigation.

5. Conclusions

This cross-sectional comparative analysis demonstrates that Alpha Guard vapor-phase hydrogen peroxide fumigation achieves significantly superior microbial contamination reduction in operation theaters compared to traditional formalin fumigation across all measured parameters:

Efficacy advantage: 15–17 percentage points superior CFU reduction with Alpha Guard

Kinetic advantage: 2.4× faster time to achieve 90% microbial reduction

Sustained efficacy: 25.1 percentage points superior sustained efficacy at 72 hours post-fumigation

Occupational health advantage: Lower formaldehyde exposure with faster aeration requirements

Implementation of Alpha Guard fumigation protocols in tertiary care operation theaters is supported by evidence of superior decontamination efficacy, faster operational kinetics, and improved occupational safety compared to formalin fumigation.

Future prospective studies examining the impact of Alpha Guard fumigation on surgical site infection rates, long-term environmental contamination surveillance, and cost-effectiveness analyses would further strengthen the evidence base for widespread adoption of this technology in surgical environments.

The findings of this study support revision of OT fumigation protocols to incorporate Alpha Guard as the preferred agent for environmental decontamination in tertiary care settings where infection prevention and operational efficiency are paramount concerns.

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