Original Article

Effects of Three Different Chemical Disinfectants on Reduction of Bacterial Load (Aerobic & Facultative) in Operation Theatres of a Tertiary Care Hospital of Punjab

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ABSTRACT

OBJECTIVE: To determine the reduction of bacterial load using three different chemical disinfectants in the operation theatres (OTs).

METHODOLOGY: This quasi-experimental study was conducted in the operation theatres of Bahawal Victoria Hospital, Bahawalpur, from February to August 2025. A total of 1260 samples were collected in three cycles using Alpha Guard, BIB Forte, or Virkon. Sampling was done from air (settle plate method), OT surfaces, and scrub areas before and after fumigation. Samples were cultured on blood and MacConkey agar and identified to genus level by biochemical tests. We analyzed data in SPSS v26 using a paired t-test, with $p<0.05$ as the significance threshold.

RESULTS: A total of 1260 samples were analyzed. Virkon achieved the greatest overall reduction (48.9%), followed by Alpha Guard (39.2%), while BIB Forte showed minimal effect (3.7%). On surfaces, Virkon reduced bacterial growth by 61.5%, BIB Forte by 25%, whereas Alpha Guard showed no overall reduction. In scrub areas, BIB Forte performed best (64.3%), compared with Alpha Guard (42.9%) and Virkon (no reduction). Statistically significant reductions in Staphylococcus spp. were observed with Virkon ($p<0.001$) and Alpha Guard ($p<0.001$), but not with BIB Forte ($p=0.056$).

CONCLUSION: *Staphylococcus spp.* was the most common bacterial isolate detected in pre- and post-fumigation samples, while Gram-negative bacteria were frequently identified in scrub areas. Virkon provided the most consistent and effective reduction in bacterial load in both air and surface samples, with Alpha Guard showing moderate but significant reductions.

KEYWORDS: Bacteria, disinfectants, fumigation, infection control, operation theatre.

INTRODUCTION

Healthcare-associated infections (HCAIs), also called hospital-acquired or nosocomial infections, are a major global concern[1]. Among HCAIs, surgical site infections (SSIs) represent about 20% of HCAIs, while 2–5% of patients undergoing surgery develop SSIs, which can result in considerable morbidity and an extension of hospital stay[2,3]. Regional data report SSIs as the 3rd most common HCAIs, accounting for 10-40% of all HCAIs[4]. A hospital-based study in Pakistan reported that nearly 4 out of 9 surgical patients developed SSIs, with those undergoing emergency procedures being disproportionately affected[5].

Commonly recommended disinfectants for healthcare settings include ethanol, chlorine, and hydrogen peroxide[6]. However, newer agents with improved safety and efficacy profiles are increasingly being adopted. Alpha Guard is a polymeric biguanide hydrochloride combined with quaternary ammonium compounds (QACs), widely used for disinfecting water - and wipe-resistant clinical surfaces, incubators, and wards[7]. BIB Forte has been widely used in hospitals but is considered less effective than newer formulations[8]. Virkon is gaining interest due to its strong bactericidal and sporicidal activity[9]. These newer disinfectants are safer alternatives to formalin and phenolic compounds, which have been phased out because of toxicity concerns[10].

Maintaining a low microbial burden in operating theatres (OTs) is crucial to minimize SSIs and ensure patient safety. The effectiveness of disinfectants may vary with chemical composition, spectrum, and procedural factors such as dilution and contact time. Alpha Guard, BIB Forte, and Virkon are commonly used disinfectants in hospital settings, yet comparative data on their effectiveness remain limited, especially from Pakistan. Documenting such evidence is important for guiding infection control practices and improving the selection of disinfectants that provide effective microbial reduction with practical comparative advantages. Therefore, this research was planned to assess and compare the efficacy of Alpha Guard, BIB Forte, and Virkon in reducing aerobic and facultative anaerobic bacterial load in OTs.

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METHODOLOGY

This quasi-experimental study was performed in OTs of Bahawal Victoria Hospital, Bahawalpur, Pakistan, from February to August 2025, following approval from the Institutional Ethical Review Committee (letter number: 28/DME/QAMC Bahawalpur, dated: 13-02-2025). A minimum sample size of 154 was calculated using the OpenEPI online calculator to detect a 10% difference in bacterial load reduction with a 95% confidence level and 80% power. To ensure reliability, of collected 210 samples in each cycle (210 before fumigation and 210 after fumigation), for a total of 1260 samples across three cycles, using one disinfectant per cycle at 3-month intervals. Sampling was performed from three sources: air, OT surfaces and equipment, and scrub areas. For air sampling, we used the settle plate method, exposing blood agar and MacConkey agar plates for 1 hour at three defined sites in each OT: near the head of the operating table, between the operating table and the wall, and near the OT entrance. Colony counts were converted into cfu/m³ using Koch's sedimentation formula. We collected surface and scrub area samples using sterile swabs, rolled clockwise and anticlockwise at predefined sites, labelled carefully, and transported promptly to the microbiology laboratory.

Before fumigation, all OTs were cleaned of removing visible debris and organic matter, cleaning of walls and floors with the three-bucket system, and wiping surfaces from clean to unclean areas. Three disinfectants were tested: Alpha Guard, BIB Forte, and Virkon. Alpha Guard was used as a 1.5% solution for surface cleaning with a 15-minute contact time and as a 0.5% solution for air fumigation with a 60-minute contact time. BIB Forte was applied at 5% for surface cleaning with a 5-minute contact time and at 10% for air fumigation with a 5-hour contact time. Virkon was used at 1% for surface cleaning and air fumigation, with contact times of 10 minutes and 2 hours, respectively. In each case, the disinfectant solution was prepared according to the manufacturer's instructions, and fumigation was performed with a ULV cold fogger (ORAMA, Italy). We fumigated elective OTs after the day's surgical list, while we fumigated emergency OTs in the morning and kept them closed for the recommended contact time. Post-fumigation samples were collected the following day before the start of surgeries in elective OTs and after the specified contact duration in emergency OTs.

All collected samples were processed in the microbiology laboratory in accordance with standard guidelines. We inoculated samples on blood and MacConkey agar and incubated plates aerobically at 35°C for 24 hours. Colony counts from air plates were recorded as cfu/m³, while surface and scrub samples were examined for bacterial growth. Gram staining was performed, and Gram-positive organisms were further evaluated using catalase and coagulase tests. In contrast, Gram-negative organisms were processed with TSI, oxidase, citrate, urease, indole, and motility tests. Data were analyzed using IBM SPSS Statistics version 26.0. Continuous variables were reported as the mean ± standard deviation (SD) or median (interquartile range [IQR]). Comparisons of pre- and post-fumigation bacterial counts were carried out using the paired-samples t-test or the Wilcoxon test, with $p<0.05$ considered significant.

RESULTS

Out of a total of 1260 samples collected, in air samples, *Staphylococcus spp.* were the most commonly isolated organisms, with pre-fumigation counts ranging from 569 cfu/m³ with Alpha Guard to 1455 cfu/m³ with Virkon. Post-fumigation, Alpha Guard reduced *Staphylococcus spp.* counts from 569 to 349 cfu/m³, reflecting a 37% reduction, while *Bacillus spp.* decreased from 52 to 27 cfu/m³ (48% reduction), and no reduction was observed in Gram-negative bacteria, resulting in an overall reduction of 39.2%. BIB Forte showed negligible effect on *Staphylococcus spp.* (849 to 844 cfu/m³, 0.5% reduction), a 42.6% reduction in *Bacillus spp.* (61 to 35 cfu/m³), and a 75% reduction in Gram-negative organisms, leading to only a 3.7% overall reduction. Virkon demonstrated the most notable effect, reducing *Staphylococcus spp.* from 1455 to 737 cfu/m³ (49.3% reduction), *Bacillus spp.* from 30 to 24 cfu/m³ (20% reduction), and Gram-negative bacteria from 6 to 1 cfu/m³ (83.3% reduction), yielding an overall reduction of 48.9% (**Table I**). In surface samples, Alpha Guard paradoxically increased Staphylococcus spp. from 19 to 32 isolates, with no reduction in Bacillus spp., but an 80% reduction in Gram-negative organisms; overall, no significant reduction was achieved. In contrast, BIB Forte reduced Staphylococcus spp. from 20 to 18 (10% reduction) and eliminated Gram-negative organisms (100%), resulting in an overall 25% reduction. Virkon again performed best, reducing Staphylococcus spp. from 23 to 9 (60.9% reduction), Bacillus spp. from 2 to 1 (50% reduction), and eliminating Gram-negative organisms, resulting in an overall 61.5% reduction (**Table I**). In scrub area samples, Alpha Guard achieved a 100% reduction in Bacillus spp., no reduction in Staphylococcus spp., and a 45% reduction in Gram-negative organisms, resulting in an overall 42.9% reduction. BIB Forte showed superior efficacy in scrub areas, with complete elimination of Staphylococcus spp. and Bacillus spp. (100% each), and a 58.3% reduction in Gram-negative organisms, leading to an overall 64.3% reduction. Virkon eliminated Staphylococcus spp. but showed no reduction in Gram-negative organisms, resulting in no overall reduction in bacterial load on scrub areas (**Table I**).

Table I: Effect of different disinfectants on different types of bacterial organisms (cfu/m^3) in samples of air, surface areas, and scrub areas of all OTs

Sample location	Disinfectant name	Fumigation status	Bacterial growth from air samples in cfu/m^3			Total
			Staphylococcus spp.	Bacillus spp.	*Gram-negative bacteria	
Air of OTs	Alpha Guard	Pre	569	52	4	625
		Post	349	27	4	380
		Reduction (%)	37%	48%	No reduction	39.2%
	BIB Forte	Pre	849	61	4	914
		Post	844	35	1	880
		Reduction (%)	0.5%	42.6%	75%	3.7%
	Virkon	Pre	1455	30	6	1491
		Post	737	24	1	762
		Reduction (%)	49.3%	20%	83.3%	48.9%
Surface areas of OTs	Alpha Guard	Pre	19	1	5	25
		Post	32	1	1	34
		Reduction (%)	No reduction	No reduction	80.0%	No reduction
	BIB Forte	Pre	20	-	4	24
		Post	18	-	0	18
		Reduction (%)	10.0%	-	100%	25.0%
	Virkon	Pre	23	2	1	26
		Post	9	1	0	10
		Reduction (%)	60.9%	50.0%	100%	61.5%
Scrub areas	Alpha Guard	Pre	2	1	11	14
		Post	2	0	6	8
		Reduction (%)	No reduction	100%	45.0%	42.9%
	BIB Forte	Pre	1	1	12	14
		Post	0	0	5	5
		Reduction (%)	100%	100%	58.3%	64.3%
	Virkon	Pre	1	-	4	5
		Post	0	-	6	6
		Reduction (%)	100%	-	No reduction	No reduction

**Gram-negative bacteria found in different air samples were Pseudomonas spp., Klebsiella spp., E. coli, and Acinetobacter spp*

For Alpha Guard, the mean *Staphylococcus spp.* counts decreased from 25.67±10.65 pre-fumigation to 10.48±9.50 post-fumigation (p<0.001), while *Bacillus spp.* decreased from 1.91±1.19 to 0.79±0.56 (p<0.001). BIB Forte showed no significant reduction in *Staphylococcus spp.* (25.67 ± 10.66 to 27.39 ± 7.44; p=0.056) but significantly reduced *Bacillus spp.* (1.91±1.19 to 1.06±0.57, p<0.001). Virkon produced the strongest effect, halving *Staphylococcus spp.* counts halved from 44.09±17.36 to 22.33±15.21 (p<0.001), while no significant difference was noted for *Bacillus spp.* (0.73±0.29 vs 0.73±0.39, p=1). (**Table II)**

Table II: Effect of fumigation with different disinfectants on growth of bacterial load

Disinfect-ant	Phase	*Staphylococcus spp.*	P-value	*Bacillus spp.*	P-value
Alpha guard	Pre	25.67±10.65	<0.001	1.91±1.19	<0.001
	Post	10.48±9.50		0.79±0.56	
Bib forte	Pre	25.67±10.66	0.056	1.91±1.19	<0.001
	Post	27.39±7.44		1.06±0.57	
Virkon	Pre	44.09±17.36	< 0.001	0.73±0.29	1
	Post	22.33±15.21		0.73±0.39	

With Alpha Guard, the greatest reduction in bacterial load was seen in gynecology OTs, falling from 91.7 to 24.3 cfu/m³ (73.5% reduction), followed by general surgery OTs (70.7% reduction), while reductions in orthopedic and emergency OTs were modest (23.5% and 45.5%, respectively). BIB Forte showed relatively little impact, with reductions of 3.9% in emergency OTs, 20% in general surgery OTs, and 30.1% in gynecology OTs, while orthopedic OTs paradoxically showed an increase in bacterial load. Virkon again showed the most consistent effect, reducing bacterial load of 71.4% in gynecology OTs, 46.3% in emergency OTs, 42.7% in orthopedic OTs, and 38.9%

in general surgery OTs (**Table III**).

Table III: Reduction in the bacterial load (cfu/m^3) from air samples of different OTs after fumigation with different disinfectants

Disinfectant Name	Fumigation status	Bacterial load in different OTs (cfu/m^3)			
		Emergency	Orthopedic	General Surgery	Gynecology
Alpha Guard	Pre	77	44	88.7	91.7
	Post	42	33.7	26	24.3
	Reduction (%)	45.5%	23.5%	70.7%	73.5%
BIB Forte	Pre	77	44	91.7	92
	Post	74	99	73.3	64.3
	Reduction (%)	3.9%	No reduction	20.0%	30.1%
Virkon	Pre	86.3	124	170.7	113
	Post	46.3	71	104.3	32.3
	Reduction (%)	46.3%	42.7%	38.9%	71.4%

DISCUSSION

In this study, *Staphylococcus spp.* was the most frequently isolated Gram-positive bacterium pre-fumigation in air, surfaces, and scrub areas, followed by *Bacillus spp.* and *Micrococcus spp.* In contrast, Gram-negative isolates included *Pseudomonas spp., Klebsiella spp., E. coli,* and *Acinetobacter spp.* Reports from Malaysia examining OT contamination reported *Bacillus spp. Micrococcus spp., and Staphylococcus spp.,* as the most commonly found isolates.[11] Recent surveillance data confirm that *Gram -positive cocci,* particularly *Staphylococcus spp.,* remain the major contaminants in healthcare environments, contributing significantly to surgical site infections[12].

This research showed that Virkon and Alpha Guard significantly reduced *Staphylococcus spp.* in air samples ($p<0.001$ and $p=0.002$, respectively), while BIB Forte had no effect ($p=0.652$). For *Bacillus spp.,* Alpha Guard ($p=0.012$) and BIB Forte ($p=0.038$) were effective, whereas Virkon showed no significant reduction. These findings reflect previously reported differences between disinfectant classes, where older QACs such as BIB Forte often underperformed newer formulations, while biguanides and peroxygen compounds exhibit more consistent bactericidal effects[13]. Recent evaluations also caution that QAC-based disinfectants may exhibit variable efficacy depending on surface type and organic load [14,15].

Alpha Guard reduced *Staphylococcus spp.,* and *Bacillus spp.* counts in air samples by 37% and 48%, respectively, yielding a 39.2% overall reduction. Regional data reported comparable results, while more recent work has confirmed the bactericidal activity of biguanides under controlled conditions.[16] BIB Forte reduced bacterial load by only 3.7%, echoing other reports where older QACs performed poorly[8,17]. By contrast, Virkon achieved an overall reduction of 48.9%, including an 83.3% decrease in Gram-negative organisms, consistent with more recent findings that peroxygen compounds such as Virkon are highly effective in reducing microbial burden in clinical environments[18].

Stratification by OT type showed that Alpha Guard was most effective in gynaecology OTs (73.5% reduction) and general surgery OTs (70.7%). In contrast, BIB Forte showed negligible effect and even increased bacterial counts in orthopaedic OTs. Virkon provided consistent reductions across all OTs, with the highest reductions in gynaecology theatres. Surface disinfection data further showed Virkon's superiority in reducing overall bacterial growth by 61.5% and eliminating Gram-negative organisms. Contemporary data have reported peroxygen compounds achieving high-level sporicidal activity[19]. In scrub areas, BIB Forte performed better. In contrast, Virkon's relatively poor performance in scrub areas highlights the critical importance of pre-cleaning, as peroxygen efficacy is reduced in the presence of organic material[20].

Another important consideration from these findings is the differential impact of disinfectants on Gram-negative organisms, which are increasingly implicated in healthcare-associated infections due to rising antimicrobial resistance. The marked reduction of Gram-negative isolates, particularly with Virkon, is clinically relevant, as organisms such as Pseudomonas spp., Acinetobacter spp., and Klebsiella spp. are well recognized for their persistence in hospital environments and their association with difficult-to-treat infections. The high susceptibility of these organisms to peroxygen-based disinfectants may be attributed to oxidative damage to cellular proteins and nucleic acids, which conventional resistance mechanisms are less likely to affect[9]. In contrast, the relatively limited efficacy of QAC-based formulations against Gram-negative bacteria observed in this study aligns with concerns regarding adaptive resistance and biofilm formation.[17] These findings underscore the importance of selecting disinfectants with broad-spectrum activity, particularly in high-risk areas such as operation theatres where environmental contamination can directly influence postoperative infection rates.

Optimal decontamination requires not only selecting appropriate disinfectants but also following pre-cleaning protocols, using correct dilutions, and ensuring adequate contact time [21]. OT design, ventilation systems, and staff discipline could play critical roles in reducing recontamination[22]. Strengthening microbial surveillance, regular fumigation, and implementation of strict prevention protocols seem essential to maintaining an aseptic OT environment and may help reduce the burden of HCAIs[23,24].

Limitations of this study include its single-centre design, modest sample size, non-probability sampling, and use of an older fumigation device, which may limit generalizability. Larger, multicenter studies with standardized protocols are needed to validate these findings.

CONCLUSION

Staphylococcus spp. was the most common bacterial isolate detected in pre- and post-fumigation samples, while Gram-negative bacteria were frequently identified in scrub areas. Among the disinfectants tested, Virkon provided the most consistent and effective reduction in bacterial load in both air and surface samples, with Alpha Guard showing moderate but significant reductions. BIB Forte demonstrated poor efficacy, particularly in air and surface disinfection, though it performed relatively better in scrub areas.

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AUTHOR CONTRIBUTION

Abbas MW: Data collection, drafting, responsible for data integrity, approved for publication
Javed I: Conception and design, proofreading, critical revisions, approved for publication
Munir S: Drafting, proofreading, critical revisions, approved for publication
Zareen A: Data collection, literature review, proofreading, critical revisions, approved for publication

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