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FORMULATION-DEPENDENT ANTIMICROBIAL PERFORMANCE OF FOUR ANTISEPTIC HAND HYGIENE PREPARATIONS AGAINST MDR NOSOCOMIAL PATHOGENS: IN VITRO MIC, MBC AND ZONE OF INHIBITION ANALYSIS

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ABSTRACT

Background: Antiseptic hand hygiene products-isopropyl alcohol (IPA) 70%, chlorhexidine gluconate (CHG) 4%, and triclosan 0.5%-are commonly stocked in Indian hospitals and diagnostic laboratories. Selection is typically based on cost and availability rather than systematic efficacy data. With multidrug-resistant (MDR) infections rising across India, evidence-based comparison of these products against resistant bacteria is urgently needed. **Objectives:** To compare the in vitro antimicrobial activity of four hand hygiene formulations-assessed by minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and zone of inhibition (ZOI)-against phenotypically confirmed MDR nosocomial isolates; and to determine whether formulation type influences outcomes against MDR organisms. **Materials and Methods:** Four antiseptic preparations were tested against 33 bacterial isolates (29 clinical + 4 ATCC quality control strains) from five nosocomial species with confirmed resistance profiles-methicillin-resistant *Staphylococcus aureus* (MRSA), ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli*, and MDR *Pseudomonas aeruginosa*. Zones of inhibition (ZOI) were measured by agar disc diffusion; minimum inhibitory concentrations (MIC) by CLSI M07:2024 broth microdilution; and minimum bactericidal concentrations (MBC) by sub-culture from MIC plates. Bactericidal activity was defined as an MBC:MIC ratio of ≤ 4. **Results:** IPA 70% demonstrated superior performance-

highest mean ZOI (22.1 ± 2.3 mm), lowest MIC (40–55% v/v), and bactericidal activity (MBC:MIC ≤ 2) against all 33 isolates including all MDR phenotypes. CHG 4% was bactericidal against susceptible organisms (MBC:MIC = 2) but only inhibitory against MRSA (MBC:MIC > 4; ZOI 11.1 ± 1.7 mm) and MDR *P. aeruginosa* (ZOI 6.2 ± 1.3 mm). Triclosan 0.5% inhibited but did not kill all MDR organisms. Plain soap showed no antimicrobial activity (ZOI = 0 mm). One-way ANOVA confirmed significant inter-formulation differences ($F[3,128] = 187.4$, $p < 0.001$); two-way ANOVA showed a significant formulation × resistance interaction ($F[3,124] = 14.2$, $p < 0.001$). **Conclusion:** IPA 70% is the only hand hygiene formulation that reliably kills bacteria regardless of resistance phenotype and should be the first-choice agent in institutional antiseptic formularies. CHG 4% retains a role in surgical antisepsis. Triclosan 0.5% should be phased out from all healthcare and laboratory formularies.

KEYWORDS: Antiseptic formulation; isopropyl alcohol; chlorhexidine gluconate; triclosan; minimum inhibitory concentration; minimum bactericidal concentration; zone of inhibition; multidrug resistance; formulary rationalisation; antiseptic stewardship.

1. INTRODUCTION

Walk into any hospital ward, clinical laboratory, or outpatient facility in India and you will almost certainly find a hand hygiene dispenser on the wall. What you are far less likely to find is documented evidence that the product inside it was selected on the basis of its proven efficacy against the bacteria circulating in that setting. Antiseptic formulations are routinely procured on grounds of cost, familiarity, or vendor availability-not on comparative microbiological data.

This matters because the active ingredient-not the brand name or the colour of the bottle-is what determines whether a hand hygiene product actually kills bacteria. IPA 70% v/v acts by physically denaturing proteins and dissolving the lipid bilayer of the bacterial cell membrane. Because this is a non-specific physical attack with no discrete molecular target, bacteria have no straightforward genetic route to resistance-a critical practical advantage.[1,2]

CHG 4% w/v works differently. It binds electrostatically to the bacterial cell membrane and disrupts it progressively, with the added benefit of residual skin adherence that sustains antimicrobial activity after application. These properties make it a valued agent in surgical antisepsis. The concern, however, is that a subset of bacteria-MRSA foremost among them-

carry *qacA/B* efflux pump genes that actively expel CHG molecules from the cell before a lethal intracellular concentration is reached.[3,4]

Triclosan 0.5% occupies a different category altogether. It targets a single enzyme-FabI (enoyl-acyl carrier protein reductase)-in the bacterial fatty acid synthesis pathway. This specificity is exactly what makes it vulnerable: a single point mutation in *fabI*, or co-expression of existing antibiotic efflux pumps such as *MexCD-OprJ* in *P. aeruginosa*, is sufficient to confer meaningful resistance. In effect, triclosan behaves more like an antibiotic than a classical biocide.[5-8]

These mechanistic differences would matter less if the organisms encountered in Indian healthcare settings were predominantly susceptible strains. They are not. MRSA now accounts for 30–40% of clinical *S. aureus* isolates nationally; ESBL-producing *K. pneumoniae* constitutes 55–62% of isolates at tertiary centres; and roughly 35% of *P. aeruginosa* isolates meet criteria for multidrug resistance (ICMR, 2022).[27-29] Against this background, the question is not merely academic: which hand hygiene products stocked in Indian institutions actually kill the bacteria causing infections there?

The present study was designed to answer that question directly. We compared the in vitro antimicrobial activity of four formulations-IPA 70%, CHG 4%, triclosan 0.5%, and plain soap-across a panel of phenotypically characterised resistant and susceptible clinical isolates, using ZOI, MIC, and MBC as complementary endpoints, with the goal of providing actionable, evidence-based guidance for institutional antiseptic formulary decisions.

2. MATERIALS AND METHODS

2.1 Ethics and Regulatory Compliance

This was a purely in vitro study conducted on de-identified archived clinical bacterial isolates; no patients were recruited and no new clinical samples were collected. Institutional biosafety precautions were observed throughout the studies.

2.2 Test Formulations

Four hand hygiene preparations were selected to represent the range of products routinely stocked in Indian hospitals and diagnostic laboratories (Table 1). These were: IPA 70% v/v, CHG 4% w/v, triclosan 0.5% soap, and plain (non-antimicrobial) soap as a negative control.

Table 1: Test formulations: active ingredient, mechanism of action, and preparation method.

Formulation	API (Use Concentration)	Mechanism of Action	Preparation
IPA 70% v/v	Isopropyl alcohol 70% v/v	Protein denaturation and cell membrane disruption (physical kill-no specific molecular target)	Analytical-grade IPA diluted to 70% v/v in sterile distilled water
CHG 4% w/v	Chlorhexidine gluconate 4% w/v	Electrostatic binding to bacterial cell membrane; residual skin adherence	Commercial CHG 4% solution (Himedia/Sigma); used undiluted
Triclosan 0.5% soap	Triclosan 0.5% w/v	Inhibits FabI (enoyl-ACP reductase), blocking fatty acid synthesis	Commercial antibacterial soap (0.5% triclosan); used undiluted
Plain soap (control)	None	Surfactant only-mechanical removal; no direct antimicrobial activity	Non-antimicrobial soap (pH 5.5–7.0); used undiluted

2.3 Test Organisms

The test panel comprised 33 organisms in total: 29 clinical isolates and 4 ATCC reference strains included for quality control (Table 2). Clinical isolates were sourced from archived stocks held at a private clinical laboratory and had been previously characterised during routine diagnostic work. Working cultures were prepared from glycerol stocks (−80°C) on Blood Agar, with passage number kept to ≤3 under BSL-2 conditions. Species-level identification was confirmed by VITEK 2 Compact automated identification (BioMérieux; confidence threshold ≥91%). Resistance phenotypes were confirmed using validated criteria: MRSA was identified by cefoxitin disc testing (CLSI M100-ED33:2023) combined with oxacillin MIC determination; ESBL production was confirmed by the Double-Disc Synergy Test (DDST); and MDR was defined using the Magiorakos *et al.* (2012) criteria, requiring non-susceptibility in at least three antimicrobial categories.[15]

Table 2: Test organism panel: species, phenotype, resistance confirmation, and rationale.

Organism	Phenotype	n	Resistance Confirmation	Rationale
S. aureus	MSSA	4	Cefoxitin zone ≥22 mm; Oxacillin MIC ≤0.25 µg/ml	Susceptible comparator for MRSA
S. aureus	MRSA	3	Cefoxitin zone 10–14 mm; Oxacillin MIC ≥8 µg/ml	Major resistant gram-positive; CHG tolerance risk
K. pneumoniae	Susceptible	3	CTX zone 28–32 mm; no DDST enhancement	Susceptible gram-negative comparator
K. pneumoniae	ESBL-producing	3	CTX zone 12–16 mm; DDST enhancement 12–13 mm	Plasmid-mediated resistance; high clinical

				impact
E. coli	Susceptible	4	CTX zone 26–30 mm; no DDST enhancement	Susceptible comparator
E. coli	ESBL-producing	4	CTX zone 13–16 mm; DDST enhancement 8–10 mm	Most common ESBL producer in Indian settings
P. aeruginosa	Susceptible	3	PIP-TAZ zone 22–24 mm; Imipenem zone 24–26 mm	Intrinsically resistant comparator
P. aeruginosa	MDR	3	Non-susceptible in ≥3 categories; Imipenem intermediate	Highest-risk gram-negative
E. faecalis	Susceptible	2	Vancomycin sensitive; HLGR negative	Biofilm-forming gram-positive
ATCC reference strains	QC (4 spp.)	4	Within CLSI M100-ED33:2023 QC ranges	Method validation

2.4 Zone of Inhibition Testing

ZOI testing was performed on Mueller-Hinton Agar (MHA; pH 7.2–7.4; standardised depth of 4 mm). Plates were inoculated using a 0.5 McFarland suspension (absorbance 0.08–0.13 at 625 nm) applied by the three-direction swabbing technique. Sterile 6 mm filter paper discs were each loaded with 20 µl of the respective test formulation and placed at equidistant positions on the inoculated agar surface. After a 15-minute drying period, plates were incubated at 35 ± 2°C for 18–24 hours. ZOI diameters were measured to the nearest 0.5 mm in reflected light using calibrated callipers. Every formulation–organism combination was tested in triplicate across three independent experimental days, and results are reported as mean ± SD. Activity was graded using the scheme proposed by Kampf (2018): 0 mm = no activity; 1–10 mm = weak; 11–20 mm = moderate; >20 mm = strong.

2.5 MIC Determination

MIC values were determined by the broth microdilution method (CLSI M07:2024) in Cation-Adjusted Mueller-Hinton Broth (CAMHB) using 96-well microtitre plates. Two-fold serial dilutions were prepared across the following concentration ranges: IPA 70–10% v/v; CHG 4–0.125% w/v; triclosan 0.5–0.03% w/v. Each well was inoculated to a final density of 5×10^5 CFU/ml and incubated at 35 ± 2°C for 18–20 hours. The MIC was defined as the lowest concentration at which no visible turbidity was observed. ATCC quality control strains were included on every plate to verify assay performance.

2.6 MBC Determination

Following MIC reading, 10 µl was sub-cultured from each turbidity-free well onto Blood Agar and incubated at 37°C for 48 hours. The MBC was defined as the lowest concentration achieving a ≥99.9% reduction in viable count (≥3 $\log_{10}$ CFU/ml) relative to the starting inoculum, following the convention of Pankey and Sabath (2004).[16] An MBC:MIC ratio of ≤ 4 was used as the threshold for bactericidal classification; ratios > 4 were classified as bacteriostatic.

2.7 Statistical Analysis

Inter-formulation differences in ZOI were assessed by one-way ANOVA followed by Tukey's honestly significant difference (HSD) post-hoc test. Differences in ZOI between susceptible and resistant isolate pairs for each formulation were evaluated using independent samples t-tests. The interaction between formulation type and organism resistance phenotype on ZOI was examined by two-way ANOVA. The relationship between CHG MIC and CHG ZOI across the isolate panel was assessed by Pearson's product-moment correlation (two-tailed; $\alpha = 0.05$). All statistical analyses were performed using IBM SPSS Statistics version 26.0. All data are presented as mean ± standard deviation.

3. RESULTS AND DISCUSSION

3.1 Overall Ranking of Formulations

The most striking finding of this study is how consistently IPA 70% outperformed every other formulation across every organism tested. A clear, reproducible hierarchy emerged across all 33 isolates: IPA 70% > CHG 4% > triclosan 0.5% > plain soap. One-way ANOVA confirmed that these differences were not due to chance ($F[3,128] = 187.4$, $p < 0.001$), and Tukey's HSD post-hoc testing established that every pairwise comparison was independently significant ($p < 0.001$). Plain soap, as expected, produced a ZOI of zero against every organism tested-a reminder that its role is physical removal of contamination, not antimicrobial killing. Representative disc diffusion plates for all four key organisms are shown in Figures 1–4.

Table 3: Mean zone of inhibition (mm ± SD) for four antiseptic formulations against all test organisms. *p < 0.001 vs. susceptible counterpart (independent samples t-test).

Organism (Phenotype)	IPA 70% (mm)	CHG 4% (mm)	Triclosan 0.5% (mm)	Plain Soap (mm)
S. aureus MSSA (n=4)	26.0 ± 2.1	22.4 ± 1.7	13.9 ± 1.3	0
S. aureus MRSA (n=3)*	24.1 ± 2.1	11.1 ± 1.7*	10.0 ± 1.3*	0
K. pneumoniae Susceptible (n=3)	22.1 ± 1.7	14.4 ± 1.3	10.6 ± 1.1	0
K. pneumoniae ESBL (n=3)*	21.0 ± 1.9	10.4 ± 1.5*	8.2 ± 1.3*	0
E. coli Susceptible (n=4)	23.1 ± 1.9	15.5 ± 1.3	11.9 ± 1.5	0
E. coli ESBL (n=4)*	21.1 ± 2.1	12.1 ± 1.7*	9.3 ± 1.1*	0
P. aeruginosa Susceptible (n=3)	20.1 ± 1.9	10.6 ± 1.3	8.2 ± 1.1	0
P. aeruginosa MDR (n=3)*	18.4 ± 2.1	6.2 ± 1.3*	5.2 ± 1.1*	0
E. faecalis Susceptible (n=2)	24.0 ± 1.9	20.4 ± 1.5	12.6 ± 1.3	0
ATCC QC (n=4), mean	24.8 ± 2.1	18.6 ± 5.2	12.8 ± 2.7	0
Overall mean	22.1 ± 2.3	14.4 ± 4.8	10.1 ± 2.4	0

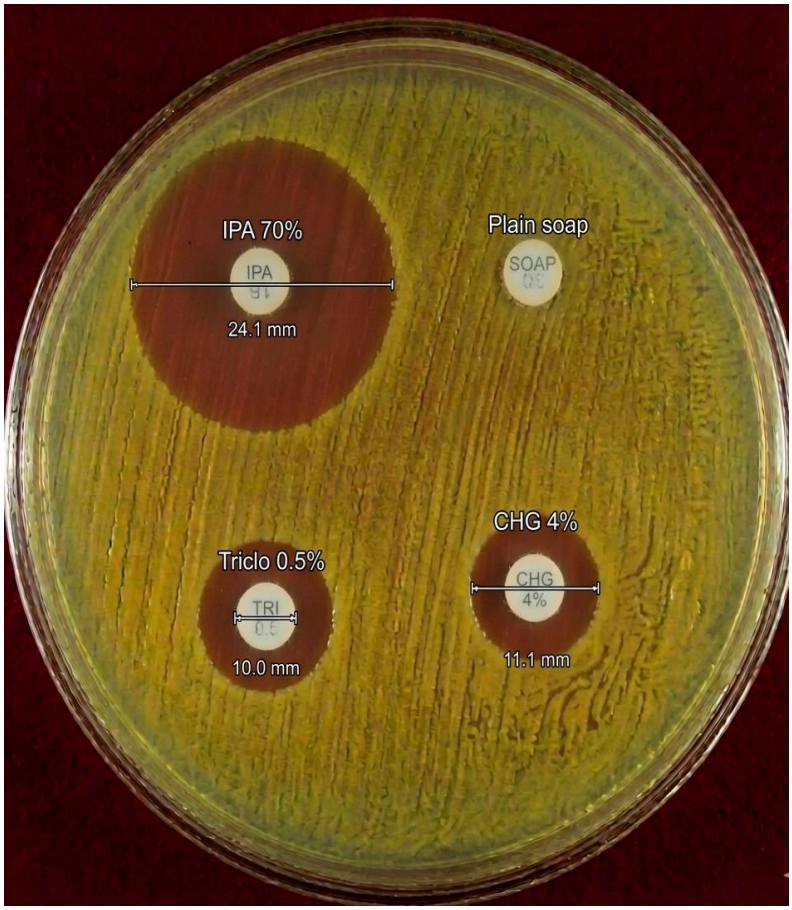

Fig. 1: Disc diffusion plate showing zones of inhibition (ZOI) of four antiseptic formulations against methicillin-resistant *Staphylococcus aureus* (MRSA) on Mueller-Hinton Agar after 18–24 h at 35 ± 2°C. IPA 70% = 24.1 mm; CHG 4% = 11.1 mm; Triclosan 0.5% = 10.0 mm; Plain soap = 0 mm (no zone). Disc diameter = 6 mm.

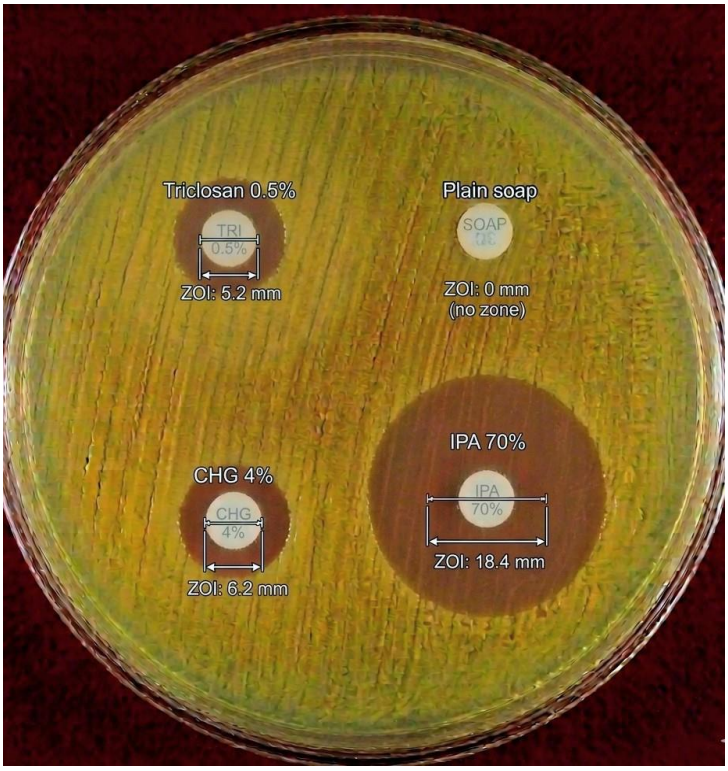

Fig. 2: Disc diffusion plate showing ZOI of four antiseptic formulations against multidrug-resistant *Pseudomonas aeruginosa* (MDR *P. aeruginosa*) on Mueller-Hinton Agar after 18–24 h at 35 ± 2°C. IPA 70% = 18.4 mm; CHG 4% = 6.2 mm; Triclosan 0.5% = 5.2 mm; Plain soap = 0 mm. Note markedly reduced CHG and triclosan zones compared to Fig. 1, consistent with MDR phenotype. Disc diameter = 6 mm.

3.2 MIC, MBC, and Bactericidal Classification

Table 4: MIC, MBC, and bactericidal classification for four formulations against susceptible and resistant organisms.

Formulation	Organism (Phenotype)	MIC	MBC	MBC:MIC	Bactericidal?
IPA 70%	MSSA / MRSA / All MDR	40–55% v/v	40–55% v/v	1	YES-all phenotypes
CHG 4%	Susceptible (all spp.)	0.25–1.0%	0.5–2.0%	≤2	YES
CHG 4%	MRSA	4% (= use conc.)	No kill at 4%	>4	NO-tolerance confirmed
CHG 4%	MDR P. aeruginosa	≥4%	No kill	>4	NO
CHG 4%	ESBL Enterobacteriaceae	2%	No kill at 4%	>4	NO
Triclosan 0.5%	Susceptible gram-positives	0.25%	1%	4	Borderline
Triclosan 0.5%	All MDR organisms	0.5% (= use conc.)	No kill	>4	NO
Plain soap	All organisms	None	None	N/A	NO

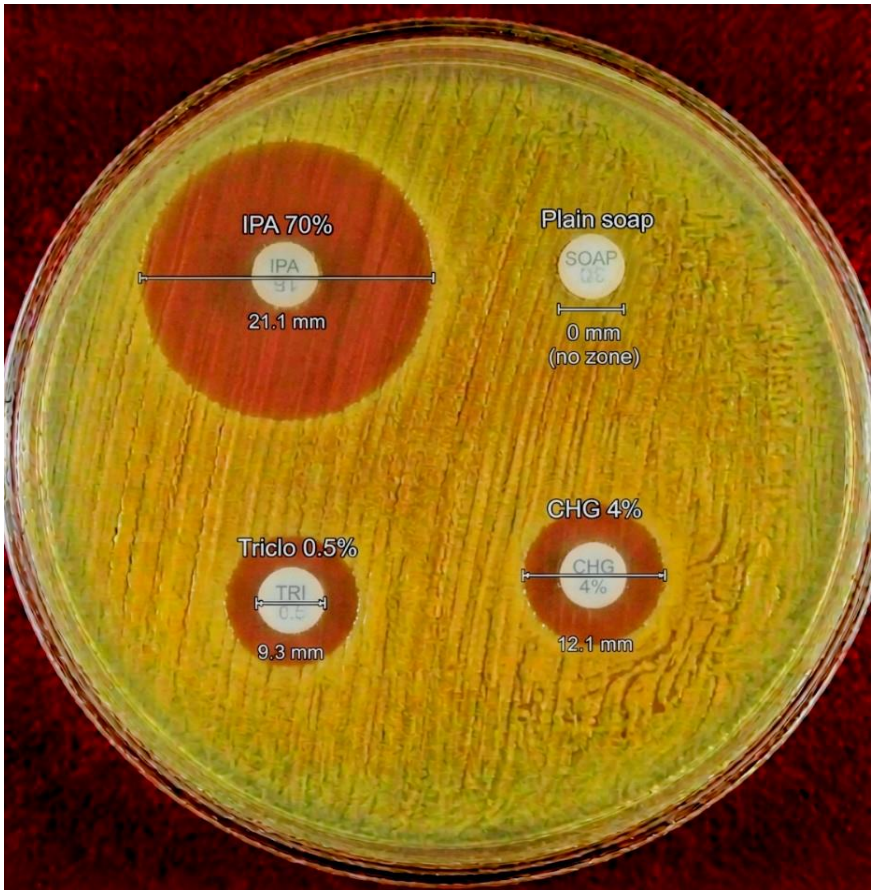

Fig. 3: Disc diffusion plate showing ZOI of four antiseptic formulations against ESBL-producing *Escherichia coli* on Mueller-Hinton Agar after 18–24 h at 35 ± 2°C. IPA 70% = 21.1 mm; CHG 4% = 12.1 mm; Triclosan 0.5% = 9.3 mm; Plain soap = 0 mm. Disc diameter = 6 mm.

3.3 Effect of Bacterial Resistance on Each Antiseptic

Perhaps the most clinically important finding in this study is that resistance does not affect all antiseptics equally. Two-way ANOVA revealed a highly significant interaction between formulation type and resistance phenotype on ZOI ($F[3,124] = 14.2$, $p < 0.001$)-in other words, the magnitude of the performance penalty imposed by MDR status depended critically on which formulation was being used (Table 5). As the plate photographs in Figures 1–3 visually demonstrate, the ZOI reduction in MDR and ESBL organisms is far more pronounced for CHG and triclosan than for IPA.

Table 5: Formulation-dependent ZOI reduction in MDR versus susceptible organisms.

Formulation (API)	Overall Mean ZOI (mm)	MDR Mean ZOI (mm)	% Reduction vs. Susceptible	Bactericidal vs. MDR?
IPA 70% (isopropyl alcohol)	22.1 ± 2.3	20.8 ± 2.2	14% ↓ ($p < 0.05$)	YES
CHG 4% (chlorhexidine gluconate)	14.4 ± 4.8	8.6 ± 2.4	40% ↓ ($p < 0.001$)	NO
Triclosan 0.5%	10.1 ± 2.4	7.6 ± 1.6	25% ↓ ($p < 0.001$)	NO
Plain soap (no API)	0	0	N/A	NO

3.4 Mechanistic Explanation for Differential Performance

IPA 70% owes its reliability to the brutally non-specific nature of its killing mechanism. Alcohol denatures proteins and dissolves the lipid bilayer of the bacterial cell membrane through physical chemistry-a process that requires no receptor, no specific binding site, and no molecular target that could be mutated away. The modest 14% ZOI reduction seen in MDR isolates is attributable to minor differences in cell wall composition, not acquired resistance.[5,12]

CHG 4% presents a more complicated picture. When it works, it works well-but in MRSA, it demonstrably does not always work at the use concentration. The 40% ZOI reduction in MDR organisms is mechanistically explained by *qacA/B*-mediated efflux: pump proteins encoded by these plasmid-borne genes actively transport CHG out of the bacterial cell, preventing lethal intracellular accumulation.[17-20] This study found a strong negative correlation between CHG MIC and CHG ZOI across the panel ($r = -0.78$; 95% CI −0.88 to −0.62; $p < 0.001$)-the higher the MIC, the smaller the zone, exactly as the efflux hypothesis predicts. Critically, in all three MRSA isolates tested, the CHG MIC equalled or exceeded the product's use concentration of 4% w/v, meaning the formulation could inhibit but not kill-a clinically meaningful failure mode that would not be apparent without comparative testing.[41]

Triclosan 0.5% performed least reliably against MDR organisms, showing a 25% ZOI reduction. The underlying mechanism-*fabI* mutation combined with cross-resistance via pre-existing antibiotic efflux pumps such as *MexCD-OprJ* in *P. aeruginosa*-is exactly what one would predict from an agent whose mechanism of action resembles that of an antibiotic.[6-11] The data from this study reinforce what the literature has long suggested: triclosan should be treated as clinically obsolete for healthcare and laboratory hand hygiene applications, particularly in settings where MDR organisms circulate.

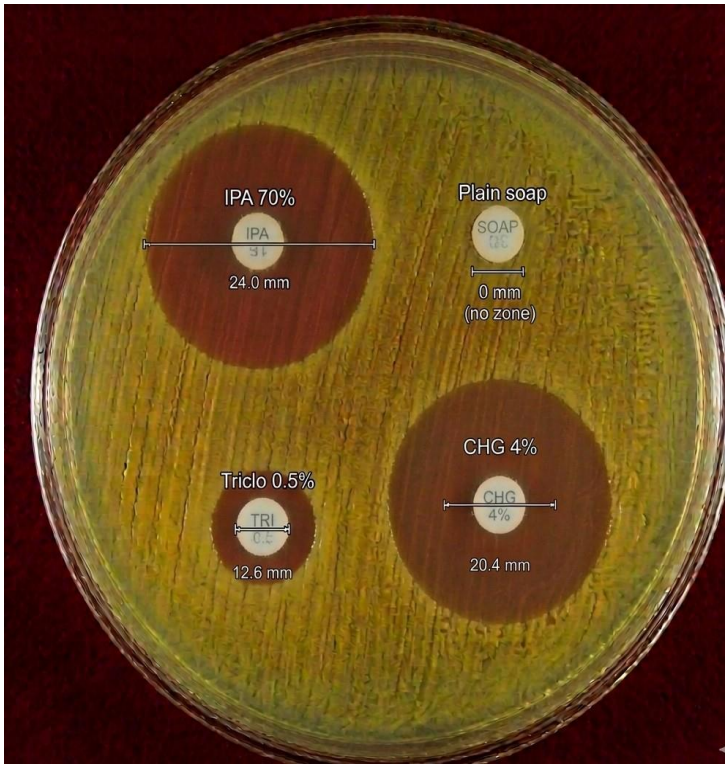

Fig. 4: Disc diffusion plate showing ZOI of four antiseptic formulations against susceptible *Enterococcus faecalis* on Mueller-Hinton Agar after 18–24 h at 35 ± 2°C. IPA 70% = 24.0 mm; CHG 4% = 20.4 mm (note large zone, nearly equivalent to IPA, contrasting sharply with CHG performance against MRSA in Fig. 1); Triclosan 0.5% = 12.6 mm; Plain soap = 0 mm. Disc diameter = 6 mm.

3.5 Practical Formulary Recommendations

Table 6: Evidence-based antiseptic formulary recommendations based on MIC, MBC, and ZOI data.

Formulation	Recommendation	Evidence Level	Action
IPA 70% v/v	First-line hand hygiene agent-all clinical settings	STRONG	Stock at all hand hygiene stations; replace CHG and triclosan for routine use
CHG 4% w/v	Second-line-surgical hand scrub and vascular access only	MODERATE	Retain in surgical formulary; remove from general-ward dispensers
Triclosan 0.5% soap	Discontinue-no bactericidal activity vs. MDR organisms	CONDITIONAL	Stop procurement immediately; replace with IPA 70%
Plain soap	Retain for visibly soiled hands prior to IPA 70% application	STRONG	Keep at stations alongside IPA 70%; not a standalone antiseptic

3.6 Limitations

Several limitations of this study deserve acknowledgement. First, in vitro ZOI and MIC data cannot be directly extrapolated to predict in vivo hand decontamination efficacy, where additional variables-contact time, formulation excipients, organic load, skin pH, and application technique-all influence outcome. Second, resistance phenotyping in this study was entirely phenotypic; while the methods used are well-validated, molecular confirmation of mecA, bla-CTX-M, and qacA/B by PCR would add an important layer of mechanistic certainty and is planned for follow-on work. Third, the clinical isolate panel (n = 29) limits statistical power for subgroup-level comparisons. Fourth, time-kill kinetics at standardised contact times-the timeframe most directly relevant to actual hand hygiene practice-would substantially strengthen the translational relevance of these findings and represent the logical next step for this research programme.

4. CONCLUSION

This study set out to ask a straightforward practical question-which hand hygiene products reliably kill the drug-resistant bacteria circulating in Indian healthcare settings?-and the answer is unambiguous: IPA 70% does, and the others do not always. IPA was the only formulation that achieved bactericidal activity (MBC:MIC $\leq$ 2) against every single isolate tested, including all MDR phenotypes, and it did so consistently. It should be the first-choice hand hygiene agent in institutional formularies.

CHG 4% retains a legitimate role in surgical hand antisepsis, where its residual activity and proven clinical impact justify its use. However, the data presented here make a strong case for removing it as the default hand rub in general wards and diagnostic laboratories where MRSA and MDR P. aeruginosa are endemic. The finding that CHG MIC equalled its use concentration in all three MRSA isolates-meaning the product inhibits but does not kill at the deployed concentration-is precisely the kind of evidence that routine procurement decisions miss.

Triclosan 0.5% soap has no defensible role in healthcare hand hygiene any longer. It failed to achieve bactericidal activity against any MDR organism at its use concentration, its mechanism is inherently vulnerable to the same efflux pumps that drive antibiotic resistance, and its continued procurement adds selective pressure without providing reliable protection. Institutions that still stock it should replace it with IPA 70%. We hope these findings

contribute to a shift-still overdue in many Indian institutions-from habit-driven antiseptic purchasing towards evidence-based formulary management.

DECLARATIONS

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REFERENCES

1. Pittet D, Allegranzi B, Boyce J. The World Health Organization Guidelines on Hand Hygiene in Health Care. Infect Control Hosp Epidemiol. 2009; 30(7): 611-622.
2. World Health Organization. WHO Guidelines on Hand Hygiene in Health Care. Geneva: WHO; 2009.
3. Boyce JM, Pittet D. Guideline for Hand Hygiene in Health-Care Settings. MMWR Recomm Rep. 2002; 51(RR-16): 1-45.
4. Sax H, Allegranzi B, Uckay I, Larson E, Boyce J, Pittet D. My five moments for hand hygiene. J Hosp Infect. 2007; 67(1): 9-21.
5. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev. 1999; 12(1): 147-179.
6. Heath RJ, Rubin JR, Holland DR, Zhang E, Snow ME, Rock CO. Mechanism of triclosan inhibition of bacterial fatty acid synthesis. J Biol Chem. 1999; 274(16): 11110-11114.
7. Heath RJ, Li J, Roland GE, Rock CO. Broad spectrum antimicrobial biocides target the FabI component of fatty acid synthesis. J Biol Chem. 1998; 273(46): 30316-30320.
8. McMurry LM, Oethinger M, Levy SB. Triclosan targets lipid synthesis. Nature. 1998; 394(6693): 531-532.
9. Schweizer HP. Triclosan: a widely used biocide and its link to antibiotics. FEMS Microbiol Lett. 2001; 202(1): 1-7.
10. Russell AD. Whither triclosan? J Antimicrob Chemother. 2004; 53(5): 693-695.
11. Chuanchuen R, Beinlich K, Hoang TT, Becher A, Karkhoff-Schweizer RR, Schweizer HP. Cross-resistance between triclosan and antibiotics in Pseudomonas aeruginosa is mediated by multidrug efflux pumps. Antimicrob Agents Chemother. 2001; 45(2): 428-432.
12. Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and inactivation with biocidal agents. J Hosp Infect. 2020; 104(3): 246-251.
13. Kampf G. Antiseptic Stewardship: Biocide Resistance and Clinical Implications. Cham: Springer; 2018.

14. Kampf G. Acquired resistance to chlorhexidine-is it time to establish an antiseptic stewardship initiative? J Hosp Infect. 2016; 94(3): 213-227.
15. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions. Clin Microbiol Infect. 2012; 18(3): 268-281.
16. Pankey GA, Sabath LD. Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of gram-positive bacterial infections. Clin Infect Dis. 2004; 38(6): 864-870.
17. Hong SI, Kim DH, Kim JY, Kwak SH, Jung SH, Kim ES, et al. qacA- and qacB-positive methicillin-resistant Staphylococcus aureus causing bloodstream infections: correlation with chlorhexidine use in ICUs. Antimicrob Agents Chemother. 2019; 63(4): e02157-18.
18. Longtin J, Seah C, Siebert L, McGeer A, Simor A, Longtin Y, et al. Distribution of antiseptic resistance genes qacA, qacB, smr in methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2011; 55(6): 2999-3001.
19. Ho CM, Li CY, Ho MW, Lin CY, Liu SH, Lu JJ. High rate of qacA- and qacB-positive methicillin-resistant Staphylococcus aureus isolates from chlorhexidine-impregnated catheter-related bloodstream infections. Antimicrob Agents Chemother. 2012; 56(11): 5693-5697.
20. Horner C, Mawer D, Wilcox M. Reduced susceptibility to chlorhexidine in staphylococci: is it increasing and does it matter? J Antimicrob Chemother. 2012; 67(11): 2547-2559.
21. Buxser S. Has resistance to chlorhexidine increased among clinically-relevant bacteria? An analysis of the literature. PLoS One. 2021; 16(8): e0256336.
22. Shukla I, Tiwari R, Agrawal M. Prevalence of extended spectrum beta-lactamase producing Klebsiella pneumoniae. Indian J Med Microbiol. 2004; 22(2): 87-91.
23. Sarojamma V, Ramakrishna V. Prevalence of ESBL-producing Klebsiella pneumoniae isolates in KIMS hospital, Bangalore. ISRN Microbiology. 2011; 2011: 318348.
24. Banerjee T, Anupurba S, Singh DK. High prevalence of multidrug-resistant Klebsiella pneumoniae from clinical isolates. Indian J Med Res. 2016; 144(4): 622-629.
25. Gill JS, Arora S, Khanna SP, Kumar HKS. Prevalence of multidrug-resistant, extensively drug-resistant, and pandrug-resistant Pseudomonas aeruginosa from a tertiary level intensive care unit. J Global Infect Dis., 2016; 8(4): 155-159.

26. Sahu MC, Padhy RN. In vitro antibacterial potency of five plants extracts against six gram-negative and gram-positive bacteria causing UTI. Asian Pac J Trop Dis. 2012; 2(2): S217-S226.
27. Walia K, Madhumathi J, Veeraraghavan B, Chakrabarti A, Kapil A, Ray P, et al. Establishing antimicrobial resistance surveillance and research network in India: journey so far. Indian J Med Res. 2019; 149(2): 164-179.
28. Gandra S, Tseng KK, Bhowmik A, Deo S, Bhatt PK, Laxminarayan R. Mortality burden of multidrug-resistant pathogens in India. Clin Infect Dis. 2019; 69(4): 563-570.
29. Indian Council of Medical Research. AMR Surveillance Network Annual Report 2021. New Delhi: ICMR; 2022.
30. Pittet D, Hugonnet S, Harbarth S, Mourouga P, Sauvan V, Touveneau S, et al. Effectiveness of a hospital-wide programme to improve compliance with hand hygiene. Lancet. 2000; 356(9238): 1307-1312.
31. Allegranzi B, Bagheri Nejad S, Combescure C, Graafmans W, Attar H, Donaldson L, et al. Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. Lancet. 2011; 377(9761): 228-241.
32. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 12th ed. CLSI standard M07. Wayne, PA: CLSI; 2024.
33. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 33rd ed. CLSI M100. Wayne, PA: CLSI; 2023.
34. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Susceptibility Tests. 13th ed. CLSI standard M02. Wayne, PA: CLSI; 2018.
35. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966; 45(4): 493-496.
36. Huang SS, Septimus E, Kleinman K, Moody J, Hickok J, Avery TR, et al. Targeted versus universal decolonization to prevent ICU infection. N Engl J Med. 2013; 368(24): 2255-2265.
37. Aiello AE, Larson EL, Levy SB. Consumer antibacterial soaps: effective or just risky? Clin Infect Dis. 2007; 45(Suppl 2): S137-S147.
38. Maillard J-Y, Pascoe M. Disinfectants and antiseptics: mechanisms of action and resistance. Nat Rev Microbiol. 2024; 22(1): 4-17.

39. Cieplik F, Jakubovics NS, Buchalla W, Maisch T, Hellwig E, Al-Ahmad A. Resistance toward chlorhexidine in oral bacteria-is there cause for concern? Front Microbiol. 2019; 10: 587.
40. Kampf G, Kramer A. Epidemiologic background of hand hygiene and evaluation of the effectiveness of agent-based hand hygiene procedures. Clin Microbiol Rev. 2004; 17(4): 863-893.
41. Kumar R, Mor R. Phenotypic Chlorhexidine Tolerance in MRSA Predicts In Vitro Antiseptic Failure: A Resistance-Stratified Efficacy Study of Hand Hygiene Agents. European Journal of Clinical Pharmacy. 2026; 8(1): 3629-3636.