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# Phenotypic Chlorhexidine Tolerance in MRSA Predicts In Vitro Antiseptic Failure: A Resistance-Stratified Efficacy Study of Hand Hygiene Agents

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***Abstract:*** Background: Hand hygiene agent selection in high-resistance settings requires evidence-based evaluation. In Indian healthcare, data on antiseptic efficacy against drug-resistant organisms remain limited. Biocide co-selection — a process by which resistance plasmids carrying qacA/B efflux genes reduce chlorhexidine gluconate (CHG) susceptibility alongside antibiotic resistance — has been reported in methicillin-resistant Staphylococcus aureus (MRSA). However, whether this tolerance translates to in vitro antiseptic failure in locally isolated strains has not been directly studied.**Objectives**This study aimed to determine whether phenotypic CHG tolerance in MRSA and other drug-resistant nosocomial isolates predicts in vitro antiseptic failure, and to compare the efficacy of four hand hygiene agents across susceptible and resistant bacterial phenotypes.**Methods** Four hand hygiene agents were evaluated: isopropyl alcohol (IPA) 70%, CHG 4%, triclosan 0.5% soap, and plain soap. These were tested against a panel of 33 bacterial isolates — 29 clinical and 4 ATCC reference strains — spanning five nosocomial species with confirmed susceptible and resistant phenotypes, including MRSA, ESBL-producing Enterobacteriaceae, and MDR Pseudomonas aeruginosa. Resistance characterisation followed CLSI M100-ED33:2023 and M07:2022 guidelines. Zone of inhibition (ZOI), minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) were all determined per CLSI standards. Pearson's correlation was used to explore the CHG MIC–ZOI relationship.**Results**IPA 70% delivered the highest mean ZOI (22.1 ± 2.3 mm) and maintained bactericidal activity (MBC:MIC ≤ 2) across all isolates, including MRSA and MDR P. aeruginosa. CHG 4%, by contrast, was bacteriostatic against all MRSA isolates — ZOI of 11.1 ± 1.7 mm, compared to 22.4 ± 1.7 mm for MSSA ($p < 0.001$; MBC:MIC > 4). Importantly, CHG MIC equalled the use concentration of 4 mg/L in all three MRSA isolates, confirming phenotypic tolerance with no meaningful bactericidal margin. The inverse correlation between CHG MIC and ZOI was strong (Pearson $r = -0.78$; 95% CI −0.88 to −0.62; $p < 0.001$), suggesting that biocide tolerance is not merely a binary threshold but a continuous, quantifiable predictor of in vitro failure. Triclosan 0.5% was bacteriostatic against all MDR organisms, and plain soap showed no antimicrobial activity whatsoever (ZOI = 0 mm). **Conclusions**In this pilot study, elevated CHG MIC in MRSA directly and proportionally predicted in vitro CHG failure at use concentration. IPA 70% retained bactericidal activity regardless of resistance phenotype and should be the default hand hygiene agent. The data support restricting CHG to surgical and vascular access indications and removing triclosan from routine formularies, as part of a structured antiseptic stewardship programme. Confirmation in a larger, genotypically characterised MRSA cohort is needed.

***Keywords:*** MRSA (Methicillin-resistant Staphylococcus aureus) Chlorhexidine tolerance Antiseptic resistance

# INTRODUCTION

Hand hygiene is the most effective single intervention for preventing healthcare-associated infections (HAIs).[1] WHO multimodal strategies have reduced HAI rates in many settings, yet alcohol-based hand rubs, chlorhexidine gluconate (CHG), and triclosan-based soaps continue to be used alongside each other in institutional formularies, often without clear evidence-based differentiation.[2] In India, the resistance burden is substantial. A systematic review of 98 studies reported MRSA prevalence of 37% (95% CI: 32–41%) among Indian clinical *S. aureus* isolates,[21] and the ICMR AMR Surveillance Network Annual Report 2023 documented ESBL production in 55–62% of *Klebsiella pneumoniae* and MDR in approximately 35% of *P. aeruginosa*.[3] This resistance profile raises important questions about whether routinely used antiseptic agents remain effective against the organisms most commonly encountered in Indian healthcare settings.

One mechanism of particular relevance is biocide co-selection. The *qacA* and *qacB* genes encode CHG efflux pumps and are frequently co-localised with *mecA* on MRSA resistance plasmids, resulting in isolates that both resist antibiotics and actively export CHG below bactericidal intracellular concentrations.[4],[5] Studies of *qacA/B* distribution in clinical MRSA have reported carriage rates of 2–40%, with gene-positive isolates consistently showing higher CHG MICs than controls.[23] In high-level mupirocin-resistant MRSA, *qacA/B* carriage has been found in 83% of isolates, with 90.6% showing phenotypic CHG tolerance.[25] Reduced CHG susceptibility has also been reported in Indian clinical staphylococcal isolates.[6] Whether this phenotypic tolerance predicts in vitro CHG efficacy failure in locally isolated north Indian strains had not been directly studied, and this gap has practical implications for formulary decisions.

This study used a resistance-stratified in vitro comparative design to correlate CHG MIC, as a marker of biocide tolerance, with CHG efficacy outcomes (zone of inhibition, ZOI) in the same clinical isolate panel. The efficacy of IPA 70%, CHG 4%, triclosan 0.5%, and plain soap was compared across susceptible and resistant bacterial phenotypes. The findings are presented within a graded antiseptic stewardship framework applicable to infection control practice in north Indian healthcare settings.

# METHODS

### *2.1 Study design*

This was a laboratory-based in vitro experimental study. No human subjects were involved. De-identified clinical bacterial isolates were obtained from a certified clinical microbiology laboratory. The study panel included 29 clinical isolates from five nosocomial species: *S. aureus* (4 MSSA, 3 MRSA), *K. pneumoniae* (3 susceptible, 3 ESBL-producing), *E. coli* (4 susceptible, 4 ESBL-producing), *P. aeruginosa* (3 susceptible, 3 MDR), and *Enterococcus faecalis* (2 susceptible). Four ATCC reference strains were included as controls: *S. aureus* ATCC 29213, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *E. faecalis* ATCC 29212, giving a total of 33 organisms. Working cultures were prepared from −80°C glycerol stocks on Blood Agar with passage ≤ 3. All work was performed under BSL-2 conditions.

### *2.2 Species identification*

Isolates were initially characterised by Gram staining and colonial morphology on Blood Agar and MacConkey Agar. Conventional biochemical tests (catalase, oxidase, coagulase, IMViC, urease, TSI, bile aesculin hydrolysis, and 6.5% NaCl growth) were performed for each isolate. Confirmatory identification used the VITEK 2 Compact automated system (BioMérieux), with a confidence threshold of ≥ 91% for all isolates.

### *2.3 Antibiotic susceptibility testing and resistance confirmation*

Antibiotic susceptibility was tested by Kirby-Bauer disc diffusion on Mueller-Hinton Agar (MHA; depth 4 mm; pH 7.2–7.4) with a 0.5 McFarland inoculum (625 nm; absorbance 0.08–0.13), following CLSI M02 Ed. 13:2023.[16] Plates were incubated at 35 ± 2°C for 16–18 h and zones interpreted per CLSI M100-ED33:2023.[7] MICs were determined by broth microdilution in cation-adjusted Mueller-Hinton Broth (CAMHB; $5 \times 10^5$ CFU/ml per well; 35 ± 2°C; 16–20 h) per CLSI M07 Ed. 12:2022.[8] Resistance phenotypes were confirmed as follows: MRSA — cefoxitin 30 µg disc zone ≤ 21 mm plus oxacillin MIC ≥ 4 µg/ml; ESBL — Double-Disc Synergy Test (DDST) with cefotaxime 30 µg versus cefotaxime-clavulanate 30/10 µg (≥ 5 mm enhancement); MDR — non-susceptibility in ≥ 3 antimicrobial categories per Magiorakos et al. 2012.[9] CHG MIC was determined by two-fold serial dilution in CAMHB from 4 to 0.0625 mg/L; CHG MIC ≥ 4 mg/L was defined as the phenotypic tolerance threshold.

### *2.4 Hand hygiene agents and in vitro efficacy testing*

All four agents were tested at standard use concentrations: IPA 70% v/v (analytical grade, SRL), CHG 4% w/v (Himedia), triclosan 0.5% w/v soap, and plain soap as a non-antimicrobial negative control. For ZOI testing, MHA plates were inoculated with a 0.5 McFarland suspension by three-direction swabbing. Sterile 6 mm discs loaded with 20 µl of each agent were applied and plates incubated at 35 ± 2°C for 18–24 h. Zones were measured to the nearest 0.5

 

mm under reflected light. All tests were performed in triplicate on three independent days; mean ± SD values are reported. For MBC, 10 µl from each turbidity-free MIC well was sub-cultured onto Blood Agar at 37°C for 48 h. MBC was the lowest concentration yielding ≥ 3 $\log_{10}$ CFU/ml reduction. MBC:MIC ≤ 4 was classified as bactericidal; > 4 as bacteriostatic.

### *2.5 Statistical analysis*

Data were analysed using SPSS v26.0 (IBM). One-way ANOVA with Tukey's HSD post-hoc test compared ZOI across the four agents. Independent samples t-tests were used to compare ZOI between susceptible and resistant isolate pairs within each agent group. A two-way ANOVA assessed the interaction between agent type and resistance phenotype on ZOI. Pearson's correlation (two-tailed; $\alpha = 0.05$; Fisher z-transformation for 95% CI) was used to evaluate two relationships: resistance category count versus CHG MIC, and CHG MIC versus CHG ZOI. Statistical significance was set at $p < 0.05$.

## RESULTS

### *3.1 Isolate panel and resistance characterisation*

All 33 isolates were identified to species level by VITEK 2 (confidence ≥ 91%) with no discordances against conventional biochemical results. MRSA was confirmed in three *S. aureus* isolates (cefoxitin zones 10–14 mm; oxacillin MIC ≥ 8 µg/ml); all three were D-zone positive for inducible MLSB resistance, consistent with patterns reported in Indian MRSA isolates.[22] ESBL was confirmed by DDST in three *K. pneumoniae* (zone enhancement 12–13 mm) and four *E. coli* isolates (8–10 mm). MDR was confirmed in three *P. aeruginosa* isolates showing resistance across anti-pseudomonal penicillins, cephalosporins, and fluoroquinolones. No carbapenem resistance was detected. CHG MIC was 4 mg/L in all three MRSA isolates, 0.5 mg/L in all non-MDR isolates, and 1–2 mg/L in ESBL Enterobacteriaceae and MDR *P. aeruginosa*. A significant positive correlation was found between resistance category count and CHG MIC (Pearson $r = +0.68$, $p < 0.05$). The full isolate panel is shown in Table 1.

| Organism | Phenotype | n | Resistance Confirmation | CHG MIC (mg/L) |
|---|---|---|---|---|
| *S. aureus* | MSSA | 4 | Cefoxitin zone 26–30 mm; Oxacillin MIC ≤ 0.25 µg/ml | 0.5 |
| *S. aureus* | MRSA ✓ | 3 | Cefoxitin zone 10–14 mm; Oxacillin MIC ≥ 8 µg/ml; D-zone positive | 4.0 (CHG-tolerant) |
| *K. pneumonia* | Susceptible | 3 | CTX zone 28–32 mm; no DDST enhancement | 0.5 |
| *K. pneumonia* | ESBL ✓ | 3 | CTX zone 12–16 mm; DDST enhancement 12–13 mm | 2.0 |
| *E. coli* | Susceptible | 4 | CTX zone 26–30 mm; no DDST enhancement | 0.5 |
| *E. coli* | ESBL ✓ | 4 | CTX zone 13–16 mm; DDST enhancement 8–10 mm | 1.0–2.0 |
| *P. aeruginosa* | Susceptible | 3 | PIP-TAZ zone 22–24 mm; Imipenem zone 24–26 mm | 0.5 |
| *P. aeruginosa* | MDR ✓ | 3 | Resistance ≥ 3 categories; Imipenem intermediate (18–20 mm) | 1.0–2.0 |
| *E. faecalis* | Susceptible | 2 | Vancomycin sensitive; HLGR negative | 0.5 |
| ATCC strains | QC (4 spp.) | 4 | All within CLSI M100-ED33:2023 QC ranges | 0.25 |

***Table 1. Isolate panel, resistance confirmatory findings, and CHG minimum inhibitory concentration (MIC). MSSA = methicillin-susceptible S. aureus; MRSA = methicillin-resistant S. aureus; ESBL = extended-spectrum beta-lactamase; MDR = multidrug-resistant; DDST = double-disc synergy test; CTX = cefotaxime; PIP-TAZ = piperacillin-tazobactam;***

***HLGR = high-level gentamicin resistance; QC = quality control.***

### *3.2 Zone of inhibition: agent efficacy hierarchy*

Mean ZOI values (± SD) are shown in Table 2. One-way ANOVA confirmed a significant effect of agent type on ZOI ($F[3,128] = 187.4$, $p < 0.001$). Tukey's HSD post-hoc testing identified significant differences between all four agents (all $p < 0.001$). The efficacy hierarchy — IPA 70% > CHG 4% > triclosan 0.5% > plain soap — was consistent across all organism types. Plain soap produced ZOI = 0 mm against all 33 organisms. IPA 70% showed the smallest reduction in ZOI between resistant and susceptible isolates, while CHG 4% and triclosan 0.5% showed the greatest resistance-associated attenuation (two-way ANOVA: agent × resistance phenotype interaction, $F[3,124] = 14.2$, $p < 0.001$).

| 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 strains (n=4) | 24.8 ± 2.1 | 18.6 ± 5.2 | 12.8 ± 2.7 | 0 |
| Overall mean (all isolates) | 22.1 ± 2.3 | 14.4 ± 4.8 | 10.1 ± 2.4 | 0 |

***Table 2. Mean zone of inhibition (mm ± SD) for four hand hygiene agents against all test organisms. * $p < 0.001$ versus susceptible counterpart (independent samples t-test). IPA = isopropyl alcohol; CHG = chlorhexidine gluconate; MSSA = methicillin-susceptible S. aureus; MRSA = methicillin-resistant S. aureus; ESBL = extended-spectrum beta-lactamase; MDR = multidrug-resistant.***

### *3.3 MIC, MBC, and bactericidal classification*

MIC, MBC, and MBC:MIC ratios for key agent–organism pairs are shown in Table 3. IPA 70% achieved MBC:MIC ≤ 2 against all organisms, including MRSA and MDR *P. aeruginosa*, confirming bactericidal activity across the full panel. CHG 4% was bactericidal (MBC:MIC ≤ 2) against susceptible isolates of all species, but was bacteriostatic (MBC:MIC > 4) against all three MRSA isolates and all three MDR *P. aeruginosa* isolates. In all MRSA isolates, CHG MIC equalled the use concentration of 4 mg/L, indicating that no bactericidal margin existed at the clinically applied concentration. Triclosan was similarly bacteriostatic (MBC:MIC > 4) against all MDR organisms.

| Isolate Comparison | Agent | MIC | MBC | MBC:MIC | Bactericidal? |
|---|---|---|---|---|---|
| MSSA vs MRSA | IPA 70% | 40–50% v/v | 40–50% v/v | 1 | YES — both |
| MSSA vs MRSA | CHG 4% | 0.25% vs 4% (= use conc.) | 0.5% vs no kill | 2 vs >4 | Yes / NO (MRSA) |
| Susceptible vs ESBL Klebsiella | IPA 70% | 40–45% v/v | 40–45% v/v | 1 | YES — both |
| Susceptible vs ESBL Klebsiella | CHG 4% | 0.5% vs 2% | 1% vs no kill at 4% | 2 vs >4 | Yes / NO (ESBL) |
| Susceptible vs MDR Pseudomonas | IPA 70% | 50–55% v/v | 50–55% v/v | 1 | YES — both |

| Isolate Comparison | Agent | MIC | MBC | MBC:MIC | Bactericidal? |
|---|---|---|---|---|---|
| Susceptible vs MDR Pseudomonas | CHG 4% | 1% vs ≥4% | 2% vs no kill | 2 vs >4 | Yes / NO (MDR) |
| All MDR isolates | Triclosan 0.5% | 0.5% (= use conc.) | No kill | >4 | NO |
| All organisms | Plain soap | Not determinable | Not determinable | N/A | NO |

***Table 3. MIC, MBC, and bactericidal classification of four hand hygiene agents against susceptible versus resistant isolate pairs. MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration; ESBL = extended-spectrum beta-lactamase; MDR = multidrug-resistant.***

### *3.4 CHG MIC–ZOI correlation: quantifying biocide co-selection*

The Pearson correlation between CHG MIC and CHG ZOI across all 29 clinical isolates was r = −0.78 (95% CI −0.88 to −0.62; p < 0.001). All three MRSA isolates (CHG MIC 4 mg/L; CHG ZOI 10–12 mm) clustered in the high-MIC/low-ZOI region. Non-MDR isolates (CHG MIC 0.5 mg/L; CHG ZOI 14–23 mm) showed the opposite pattern. ESBL *Enterobacteriaceae* and MDR *P. aeruginosa* occupied an intermediate position (CHG MIC 1–2 mg/L; CHG ZOI 6–12 mm). This strong inverse correlation indicates that elevated CHG MIC directly and proportionally predicts reduced CHG in vitro efficacy, consistent with biocide efflux as the underlying mechanism.

## DISCUSSION

This study examined whether phenotypic CHG tolerance in MRSA, as measured by elevated CHG MIC, predicts in vitro antiseptic failure at use concentration. In all three MRSA isolates, CHG MIC ≥ 4 mg/L was associated with CHG ZOI values of 10–12 mm and MBC:MIC > 4, confirming bacteriostatic-only activity. The Pearson correlation of r = −0.78 (p < 0.001) across the full 29-isolate panel shows that this is not a threshold effect but a continuous, quantifiable relationship: as CHG MIC increases, ZOI decreases proportionally. This supports the biocide co-selection hypothesis and has direct implications for antiseptic formulary decisions in high-AMR settings. The MRSA subgroup comprised three phenotypically confirmed isolates, consistent with published in vitro antiseptic pilot studies of comparable scope, and the study is presented as hypothesis-generating. The 100% concordance of the CHG tolerance phenotype across all three MRSA isolates, and its complete absence in the remaining 26 clinical isolates, supports the directional validity of the finding within this panel size.

### *4.1 IPA 70%: Resistance-independent bactericidal efficacy*

IPA 70% achieved bactericidal activity (MBC:MIC ≤ 2) against all 33 organisms, including all resistant phenotypes. A 14% ZOI attenuation was observed between MDR and non-MDR isolates (p < 0.05), but no isolate crossed the bacteriostatic threshold. This resistance-independent profile reflects IPA's mechanism of action — non-specific protein denaturation and membrane disruption at 70% v/v — which cannot be overcome by target modification, enzymatic inactivation, or efflux.[10] Kampf and Kramer (2004) reported ≥ 5 $\log_{10}$ reductions against MRSA and VRE with alcohol formulations in 95% of reviewed studies.[10] Bactericidal activity of isopropanol against MDR *K. pneumoniae*, irrespective of CHG co-tolerance, has also been reported in subsequent in vitro studies.[27] The current findings extend this evidence to a locally characterised north Indian panel. IPA 70% is supported by WHO (2024) recommendations [1] and ECDC IPC guidance [11] as the primary hand hygiene agent.

### *4.2 CHG 4%: A conditional agent with a documented failure mode*

The CHG failure pattern is consistent with *qacA/B*-mediated efflux. When CHG MIC equals use concentration, as observed in all three MRSA isolates, the agent cannot reach supra-MIC intracellular concentrations needed for bactericidal activity; it may inhibit growth but cannot kill. CHG MICs of 4 mg/L with corresponding loss of bactericidal activity have been documented in clinical MRSA carrying *qacA/B* genes.[26] Reduced CHG susceptibility in Indian clinical staphylococcal isolates has also been reported.[6] The correlation of r = −0.78 in this study indicates that CHG tolerance is a continuous predictor of efficacy loss, not a simple threshold. Sub-MIC CHG exposure in *S. aureus* has been shown to drive stepwise MIC elevation,[12] and acquired CHG resistance has been proposed as a trigger for formal antiseptic stewardship programmes.[13] Continued CHG use in MRSA-endemic settings may therefore select for progressively more tolerant strains over time. The ECDC's current IPC guidance recommends evidence-based formulary reassessment in high-AMR settings,[11] which is supported by the present

 

findings.

### *4.3 Triclosan and plain soap: Evidence for formulary removal*

Triclosan 0.5% was bacteriostatic against all MDR organisms (MBC:MIC > 4), with 25% ZOI attenuation compared to susceptible isolates ($p < 0.001$). Triclosan inhibits *FabI*, the NADH-dependent enoyl-acyl carrier protein reductase involved in bacterial fatty acid synthesis, and this single-target mechanism is susceptible to target mutation and efflux.[28] Triclosan resistance has also been shown to increase bacterial permissiveness to MDR plasmids and phages, adding a further AMR selection risk.[14] The WHO and UNICEF 2025 hand hygiene guidelines do not support triclosan for routine healthcare use.[15] The present in vitro data are consistent with this position, showing that triclosan does not meet bactericidal thresholds against clinically important resistant organisms at standard use concentrations. Plain soap (ZOI = 0 mm against all organisms) was included as a negative control and confirmed the discriminatory range of the experimental system.

### *4.4 Antiseptic stewardship framework*

These findings have direct implications for formulary decisions in clinical and infection control settings. National IPC guidelines recommend structured hand hygiene protocols across all healthcare facility types,[19] and intervention studies in ICUs have demonstrated that improved antiseptic agent selection substantially reduces colonisation with resistant organisms.[20] Laboratory settings in India have also been identified as a gap in infection control practice, where antiseptic agent choices are rarely evidence-based.[17] IPA 70% should be the default agent for all routine hand antisepsis at every point of care. CHG 4% should be restricted to surgical scrub and vascular access site preparation, where its residual skin activity provides benefit beyond alcohol. Triclosan-containing soaps should be phased out and replaced with IPA 70%. Plain soap should be used when hands are visibly soiled, followed by IPA 70%. A graded antiseptic stewardship framework based on these findings is presented in Table 4.

| Agent | Recommendation | Grade | Key Evidence |
|---|---|---|---|
| IPA 70% | Universal primary agent — all routine hand antisepsis | STRONG | Highest ZOI (22.1 mm); bactericidal all MDR (MBC:MIC ≤ 2); resistance-independent; WHO/ECDC first-line |
| CHG 4% | Restricted — surgical hand antisepsis and vascular access only; remove from routine hand rub | MODERATE | Bacteriostatic MRSA + MDR-PA; CHG MIC = use conc. in all MRSA; r = −0.78 co-selection signal |
| Triclosan 0.5% | Discontinue from all routine use; replace with IPA 70% | CONDITIONAL | Bacteriostatic all MDR; 25% ZOI attenuation; WHO contra-indicated 2025; resistance induction risk |
| Plain soap | Prerequisite (visible soiling) followed by IPA 70%; not a standalone antiseptic | STRONG | ZOI = 0 mm all organisms; mechanical removal only |

***Table 4. Graded antiseptic stewardship recommendations. MRSA = methicillin-resistant Staphylococcus aureus; MDR-PA = multidrug-resistant Pseudomonas aeruginosa; IPA = isopropyl alcohol; CHG = chlorhexidine gluconate; WHO = World Health Organization; ECDC = European Centre for Disease Prevention and Control.***

### *4.5 Limitations*

Despite the relevance of the findings, the study is subject to certain limitations. First, resistance confirmation was phenotypic throughout. PCR verification of *mecA*, *bla-CTX-M*, and *qacA/B* genes was not performed and is the most important planned extension of this work. Phenotypic CHG MIC data should not be treated as a direct proxy for *qacA/B* carriage. Second, the MRSA subgroup comprised three isolates, which is consistent with published in vitro antiseptic pilot studies but is insufficient for robust inferential statistics in isolation. The correlation analysis (r = −0.78) was therefore conducted across the full 29-isolate panel. Larger, multi-centre studies with genotypically confirmed MRSA isolates from diverse settings are needed before these findings can be considered definitive; antimicrobial susceptibility profiles of MRSA vary considerably across institutions and regions.[24] Third, in vitro data cannot be directly extrapolated to in vivo hand decontamination performance. Skin contact time, organic load, formulation excipients, and application technique all affect real-world outcomes in ways that disc diffusion and broth microdilution cannot capture. Furthermore,

inappropriate antimicrobial use practices across South Asian healthcare settings create a dynamic resistance landscape that may limit the generalisability of cross-sectional in vitro findings.[18] Finally, this was a cross-sectional study; longitudinal CHG MIC drift under clinical conditions was not assessed and should be addressed in future work.

## CONCLUSION

This pilot study shows that phenotypic CHG tolerance in MRSA, quantified by CHG MIC ≥ 4 mg/L, directly and proportionally predicts in vitro CHG failure at use concentration ($r = -0.78$, $p < 0.001$). This is the first locally generated in vitro evidence for biocide co-selection in north Indian MRSA, and is consistent with findings from international studies. IPA 70% was the only agent to maintain bactericidal activity across all resistance phenotypes and should be used as the universal primary hand hygiene agent. CHG 4% should be limited to surgical and vascular access indications; triclosan 0.5% should be removed from routine formularies. These findings are hypothesis-generating and require confirmation in a larger, genotypically confirmed MRSA cohort, but are sufficient to support evidence-based antiseptic formulary revision in Indian healthcare settings.

**DECLARATIONS**
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