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# Antiseptic Efficacy Against Wound Pathogens: Evidence for Rational Use

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Wound infections caused by multidrug-resistant bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* pose a major global health challenge, particularly in low-resource settings where antiseptics remain frontline interventions. This study evaluated the in vitro efficacy of commonly used antiseptics, povidone-iodine, hydrogen peroxide, and isopropyl alcohol, against bacterial strains isolated from infected wounds, using agar well diffusion and minimum inhibitory concentration (MIC) assays. Ciprofloxacin served as the control antibiotic. Results revealed that hydrogen peroxide demonstrated the highest overall efficacy, producing significant inhibition zones across all three isolates, with the lowest MIC values (0.125–0.75%). Isopropyl alcohol showed selective activity, effectively inhibiting *P. aeruginosa* (31.0 ± 3.61 mm, MIC 8.75%) but demonstrated no activity against *S. aureus* and *E. coli.* Povidone-iodine, at 2% concentration, was largely ineffective, Ciprofloxacin showed potent inhibition against *S. aureus* and *P. aeruginosa (*32.0 ± 4.24 mm and 14.0 ± 5.66 mm respectively) but weaker activity against *E. coli*. Statistical analysis confirmed significant differences in antiseptic performance ($p < 0.05$). These findings highlight hydrogen peroxide as the most reliable agent among those tested, while povidone-iodine requires higher concentrations or longer exposure times for effective use. The study shows the need for regular evaluations of antiseptic performance to ensure evidence-based selection of agents for effective wound care and infection prevention.

**Keywords:** Antimicrobial resistance, Antiseptics, *Escherichia coli,* Hydrogen peroxide, Wound infection

## 1. Introduction

Wound infections remain a significant clinical challenge, as they frequently prolong hospital stays, slow healing, and elevate healthcare costs. A 2024 German study found that nosocomial infections extended hospitalization by roughly 10 additional days, costing hospitals in excess of €4,000 per patient due to foregone revenue alone (Asegu *et al.,* 2024).

Recent data from Nigeria and sub-Saharan Africa confirm that healthcare-associated infections (HAIs), including wound infections, significantly prolong hospital stays and impose heavy economic costs. Prospective and cohort studies indicate that HAIs add between approximately 4 and over 13 extra days in the hospital, varying by infection type, such as bloodstream or surgical-site infections, and by healthcare setting (Fenny *et al*., 2020; Dayyab *et al.,* 2018). For example, a prospective cohort study in Ethiopia found infected patients stayed an average of 18.9 days compared to 10.6 days for uninfected patients—an excess of roughly 8.3 days (Gidey *et al.,* 2023). Broader country-level analyses suggest a substantial economic impact: according to a WaterAid report (2024), HAIs in seven sub-Saharan African countries (including Nigeria) resulted in losses equivalent to 0.4%–2.9% of GDP in 2022 when accounting for treatment expenses, lost productivity, and mortality. In Nigeria, HAI prevalence rates vary widely by hospital and ward, ranging from around 2.6% to over 20%, and recent studies report increased costs for patients and health systems due to antimicrobial resistance and HAIs, especially in tertiary hospitals (Otieku *et al*., 2023; Iliyasu *et al*., 2018).

The colonization of wounds by pathogens, especially *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, continues to complicate recovery and delay tissue repair if not managed effectively. While classic studies showed this trend, recent literature confirms its persistence in chronic and acute wound cases. Wound infections trigger

  

local and systemic host responses through microbial invasion. The presence of biofilms, a feature of chronic wounds, further reduces susceptibility to antimicrobials and hinders healing. With rising global antibiotic resistance, topical antiseptics are increasingly vital in wound care, offering broad-spectrum effectiveness and lower risk of resistance development (Gryson *et al*., 2023)

Among commonly used topical agents, Povidone-iodine (PVP-I) has demonstrated potent anti-biofilm activity (Gryson *et al*., 2023).. In vitro tests show rapid eradication of *S. aureus* and *P. aeruginosa* biofilms within 15 minutes, outperforming alternatives like polyhexamethylene biguanide (PHMB) and silver acetate (Gryson *et al.,* 2023). Hydrogen peroxide and chlorhexidine, alone, show limited ability to fully clear bacterial biofilms. However, combinations of PVP-I and $H_2O_2$ exhibit synergistic effects, significantly reducing *S. aureus* biofilm biomass and viability. Chlorhexidine has also been confirmed superior to PVP-I in preventing surgical site infections, with a 2023 meta-analysis showing an approximately 25% reduction in overall surgical site infections (SSI) risk compared to PVP-I (Bai *et al*., 2023).

Despite the routine application of antiseptics in healthcare and home settings, bacterial wound infections remain a persistent clinical challenge. This challenge is further intensified by the growing resistance of microbes to these antiseptic agents. According to the World Health Organization's 2024 findings antimicrobial resistance is now among the top 10 global public health threats, with nearly 4.95 million deaths associated with bacterial AMR in 2019, including 1.27 million deaths directly attributable to it (WHO, 2024). WHO also highlighted that resistance will continue to rise across all regions. This threatens the efficacy of common treatments for infections, including surgical and wound care. The practical use of antiseptics without laboratory-based assessment of their efficacy against prevalent wound pathogens in specific localities limits the reasonable use of these agents.

In Nigeria and numerous other areas where access to medical care, health facilities, trained personnel, medicines, or technology is limited or insufficient to meet the population's needs, there is a lack of current and region specific information regarding the effectiveness of widely used antiseptics against bacteria found in infected wounds. This deficiency in data undermines the implementation of evidence based approaches to wound care and infection control. Therefore, it is essential to conduct localized, in vitro assessments of antiseptic agents against local bacterial isolates to generate evidence that will guide doctors and nurses on which antiseptics actually work best in that specific context.

Given variable microbial susceptibility, concentrations, and contact times, regular in vitro assessments are essential to ensuring optimal antiseptic use in clinical settings. This study aims to evaluate the in vitro antimicrobial efficacy of selected antiseptics (Povidone-Iodine, Hydrogen Peroxide and Isopropyl Alcohol) against bacterial isolates from infected wounds and to suggest a more effective and rational way to use antiseptic agents in wound care.

## 2. Materials and Methods

### 2.1 Drugs, Chemicals and Culture Media

Ciprofloxacin (5 mg/mL) was the drug used. The antiseptics tested included hydrogen peroxide, povidone-iodine, and 70% isopropyl alcohol solutions. The culture medium used was Mueller-Hilton agar (Oxoid, UK) and Mueller-Hilton broth (Oxoid, UK).

### 2.2. Test Isolates

This study employed a laboratory-based experimental design to evaluate the in vitro efficacy of commonly used antiseptics against clinical isolates implicated in wound infections. Confirmed clinical isolates of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* obtained from wound samples collected from the microbiology unit of Central hospital, Agbor Delta State, were selected based on prevalence in wound infections (Leaper *et al.,* 2015).

### 2.3 Preparation of Antiseptics

Commercially available formulations of Povidone-Iodine (10%), Hydrogen Peroxide (6%) and Isopropyl Alcohol (70%), were used**.** Each antiseptic was used at its working concentration as per manufacturer's instructions.(DermNetNZ, 2023; UNL Environmental Health and Safety, 2024; CDC, 2023)

### 2.4 Standardization of inocula

Each isolate was sub-cultured and standardized to 0.5 McFaland standard using Mueller-Hilton broth by transferring a loopful of the isolates into 3-4 mL of normal saline followed by proper shaking. The turbidity of the suspension was matched with that of 0.5 McFarland standards for sensitivity test as described by Clinical and Laboratory Standards Institute (CLSI) (2021).

### 2.3 In Vitro Susceptibility Testing

#### 2.3.1 Agar Well Diffusion Method

The antiseptic efficacy was evaluated using the agar well diffusion method as described by CLSI

 

guidelines (CLSI, 2021) with slight modifications. Bacterial suspensions were prepared in normal saline to match the 0.5 McFarland standards (~1.5 × $10^8$ CFU/mL). Mueller-Hinton agar plates were inoculated by swabbing uniformly in three directions. Wells of 6 mm diameter were punched in the agar using sterile cork borer and filled with 100 µL of each antiseptic solution. Then, Ciprofloxacin (5 mg) was used as positive control for comparison and sterile distilled water was used as negative control to ensure that there was no contamination. Finally, plates were incubated at 37°C for 24 hours. Zones of inhibition were measured in millimeters using a ruler. The size of the inhibition zone was used as an indicator of antimicrobial efficacy with each test performed in triplicate.

### 2.3.2 Minimum Inhibitory Concentration (MIC) Determination

This was carried out by agar dilution method as described by Buzugbe *et al.,* (2015). Briefly, stock solutions of 10%, 6%, 70% of providone iodine, hydrogen peroxide and isopropyl alcohol were prepared. Two-fold serial dilution of each of the stock was carried out to obtain 5%, 2.5%, 1.25% and 0.625% for providone iodine, 3%, 1.5%, 0.75% and 0.375% for hydrogen peroxide and 35%, 17.5%, 8.75% and 4.375% for isopropyl alcohol respectively. Various volumes of the antiseptics were pipetted into the empty sterile petri dishes and the molten agar maintained at $40^0$ C was then added such that the concentration of the antiseptics in the various plates was accordingly as indicated above. The varying volumes of the stock solution were added into the petri dishes labeled as described above. Thereafter, the required volume of agar was added to each plate to make up a total volume of 10 mL. The plates were swirled slightly to allow even mixture of the extract and the molten agar. Each of the plates was divided into three segments from the reverse side and a loop-full of the standardized suspension of each test bacteria was streaked on the different segments labeled accordingly. The plates were incubated for 24 hours at $37^0$ C. At the end of the incubation period, the plates were read and the lowest concentration showing no visible growth was recorded as the MIC.

### 2.4 Data Analysis

All tests were performed in triplicate to ensure reproducibility and accuracy. Data were recorded as mean values ± standard deviation. The results were statistically analyzed using Tukey's post hoc test to assess significant differences between the antiseptics. A p-value of less than 0.05 was considered statistically significant. Statistical analysis was conducted using SPSS version 25.

## 3. Results and Discussion

### 3.1 Results

The antimicrobial effects of Povidone-Iodine (10%), Hydrogen Peroxide (6%), Isopropyl Alcohol (70%), and the antibiotic Ciprofloxacin were assessed against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Zones of inhibition and minimum inhibitory concentrations (MICs), were determined, with all results reported as the mean ± SD from triplicate experiments. Differences in efficacy among the treatments were evaluated using statistical analysis (ANOVA with post hoc tests). Ciprofloxacin served as a reference antibiotic to provide a benchmark for comparing the relative performance of the antiseptic agents. The results are illustrated in Plates A to C and Tables 1 and 2.

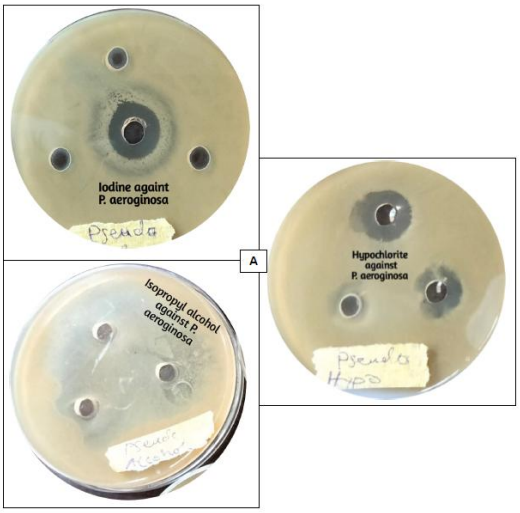


**Plate A:** Petri dishes showing the zone of inhibition against *P. aeruginosa.*

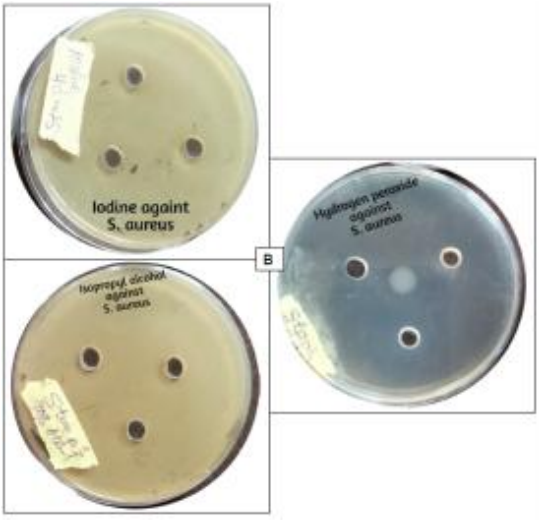


**Plate B:** Petri dishes showing the zone of inhibition against *S. aureus.*

 

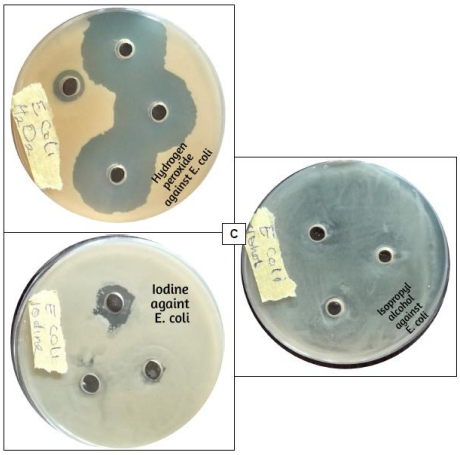


**Plate C:** Petri dishes showing the zone of inhibition against *E. coli.*

From the result obtained for the susceptibility test (Table 1), Isopropyl Ethanol was only effective against *P. aeruginosa* (zone diameter of 31.0 ± 3.61 mm and MIC at 8.75%), Povidone Iodine was ineffective at test range (2%) against the text isolates. The most effective antiseptics (lowest MICs) are Hydrogen Peroxide, andIsopropyl ethanol showed narrow spectrum (only active against *P. aeruginosa*). Povidone iodine was largely ineffective. Ciprofloxacin was potent against the test isolates, but some isolates (e.g., *P. aeruginosa*) required higher concentrations.

**Table 1:** Inhibition zone diameter (mm) and MIC of antiseptics and ciprofloxacin against bacterial isolates

| Bacteria (Test Isolates) | Treatment | Mean ± SD (mm) | MIC |
|---|---|---|---|
| ***Pseudomonas aeruginosa*** | Isopropyl Ethanol | 31.0 ± 3.61 | 8.75% |
| | Hydrogen Peroxide | 26.67 ± 1.53 | 0.125% |
| | Povidone Iodine | 0.0 ± 0.0 | 0.00% |
| | Ciprofloxacin | 14.0 ± 5.66 | 0.625 mg/mL |
| ***E. coli*** | Isopropyl Ethanol | 0.0 ± 0.0 | 0.00% |
| | Hydrogen Peroxide | 33.0 ± 1.0 | 0.75% |
| | Povidone Iodine | 10.33 ± 3.09 | 2% (no lower effect) |
| | Ciprofloxacin | 10.0 ± 3.0 | 0.3125 mg/mL |
| ***S. aureus*** | Isopropyl Ethanol | 0.0 ± 0.0 | 0.00 |
| | Hydrogen Peroxide | 23.67 ± 17.76 | 0.75% |
| | Povidone Iodine | 0.0 ± 0.0 | 0.00 |
| | Ciprofloxacin | 32.0 ± 4.24 | 0.3125 mg/mL |

Table 2: ANOVA Results for isopropyl alcohol, hydrogen peroxide, providone iodine vs ciprofloxacin

| Organism | F-statistic | p-value | Significant (p < 0.05) |
|---|---|---|---|
| **Pseudomonas aeruginosa** | 64.62 | 0.0 | True |
| **E. coli** | 99.94 | 0.0 | True |
| **S. aureus** | 7.0 | 0.0163 | True |

The result of the one way analysis of variance (ANOVA) (Table 2) showed that there is a significant difference in inhibition zones among the antiseptics and ciprofloxacin for all three isolates. The Post Hoc analysis of the antiseptics against ciprofloxacin showed that Ciprofloxacin had significantly different effects against *P. aeruginosa* compared to all antiseptics. Its inhibition of *E. coli* was significantly weaker than hydrogen peroxide and not different from the inhibition from providone iodine. However, against *S. aureus*, Ciprofloxacin significantly outperformed Isopropanol and Providone Iodine but not Hydrogen Peroxide.

### 3.2 Discussion

The study evaluated the in vitro antimicrobial efficacy of commonly used antiseptics, isopropyl ethanol, hydrogen peroxide, povidone-iodine, and a control antibiotic (ciprofloxacin), against three bacterial isolates - *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus,* known to be implicated in wound infections.From the results obtained, it was observed that hydrogen Peroxide exhibited the *highest overall efficacy*, with inhibition across all three test organisms and the *lowest MICs*, indicating strong potency even at low concentrations.

The study's findings indicated that hydrogen peroxide exhibited the most extensive spectrum and potent antimicrobial efficacy compared to all other agents evaluated. It generated wide zones of inhibition against every isolate, with the most pronounced effect observed against *E. coli* (33.0 ± 1.0 mm). Its low Minimum Inhibitory Concentration (MIC) values, ranging from 0.125% to 0.75%, indicate that it retains high potency even when significantly diluted. This robust performance is explained by hydrogen peroxide's oxidizing mechanism of action: it produces reactive oxygen

 

species (ROS) like hydroxyl radicals that induce oxidative damage to critical bacterial structures, including lipids in the cell membrane, proteins, and DNA. This inflicts oxidative stress that bacterial defense mechanisms are frequently unable to overcome at low antiseptic concentrations (Brudzynski et al., 2011; ScienceDirect, 2024). The strong antimicrobial activity of hydrogen peroxide in this study equally aligns with findings by Finnegan *et al.* (2010)**,** who also reported that hydrogen peroxide's efficacy is linked to its ability to generate reactive oxygen species that cause wise-ranging oxidative damage to bacterial cells. The observed performance of hydrogen peroxide in this study is supported by its ability to act broadly across Gram-negative and Gram-positive bacteria. These results underscore hydrogen peroxide's utility as a powerful, cost-efficient, and readily accessible antiseptic, making it highly appropriate for broad-spectrum wound disinfection.

From the susceptibility testing, Isopropyl Ethanol demonstrated selective antimicrobial activity. It was effective only against *Pseudomonas aeruginosa* (31.0 ± 3.61 mm; MIC = 8.75%) but showed no inhibition of *E. coli* or *S. aureus*. This selective activity could be due to structural differences in bacterial cell walls and membranes. Gram-negative *Pseudomonas* might be more susceptible to alcohol-mediated membrane disruption, whereas the resistance observed in *E. coli* and *S. aureus* may be linked to their cellular resilience and protective outer layers. This finding emphasizes that while isopropyl alcohol is often used as a disinfectant, its efficacy is limited against certain wound pathogens. The narrow effect exhibited aligns with previous findings that ethanol is more effective against Gram-negative bacteria due to differences in cell wall structure and lipid composition (McDonnell & Russell, 1999). Luther *et al.* (2015) found that exposure to >40% ethanol or isopropyl alcohol actually increases biofilm formation in *S. aureus* and *S. epidermidis*, with viable cells persisting within these biofilms. Similarly, Oraziand O'Toole (2019) demonstrated that ethanol at concentrations used clinically failed to reduce viability of *S. aureus* biofilms, even in the presence of *P. aeruginosa*.

However, newer findings support alcohol's specific action against *P. aeruginosa*. Lewis *et al.* (2019) showed that low ethanol concentrations impaired *P. aeruginosa* swimming motility, up to a 45% reduction, hinting at a potential anti-biofilm benefit. And Hamad *et al***.** (2022) confirmed the strong antimicrobial activity of alcohol-based sanitizers against *P. aeruginosa* in vitro.

The apparent failure of isopropyl alcohol to inhibit *E. coli* and *S. aureus* can reflect rapid evaporation and inadequate wet contact time on surfaces, alongside biofilm-associated tolerance that reduces susceptibility to biocides; recent work shows alcohol components may evaporate before achieving required log-reductions, (Voorn *et al*., 2023; Copes and Smith, 2024).

Povidone-iodine, despite being a widely used antiseptic in clinical practice, was largely ineffective, showing zero or minimal inhibition at the tested concentration. It had no inhibition against *P. aeruginosa* and *S. aureus* and only weak inhibition against *E. coli*. The ineffectiveness may stem from the low concentration (2%), which might be below the threshold required for bactericidal activity. In clinical settings, higher concentrations (e.g., 5–10%) are often employed (Bigliardi et al., 2017; Pichon *et al*., 2022). These results suggest that low concentrations may not provide adequate wound antisepsis, especially against multidrug-resistant organisms. . The poor performance of povidone-iodine at the tested concentration is in line with the findings of Bigliardi *et al*., (2017) who noted that the efficacy of Povidone-Iodine can be compromised by organic matter and short exposure times**.** In this case, a 2% solution had minimal to no observable effect, showing the need for higher concentrations or longer contact duration in clinical use. A more recent study confirms that even at moderate concentrations, such as 7.5%, PVP-I exhibits significantly reduced antimicrobial activity in the presence of organic load, underscoring the necessity for either higher concentrations or prolonged contact times in clinical settings for reliable disinfection. (Şahiner *et al*., 2022)

In contrast, more recent research continues to support povidone-iodine's rapid and broad-spectrum bactericidal activity against Gram-positive organisms. For example, a 2025 in vitro study demonstrated that a 0.5% povidone-iodine solution achieved a ≥5-log reduction in *S. aureus* (ATCC 25923) within just one minute, while 0.25% concentrations were effective against *Klebsiella aerogenes* within 5 seconds (Lacuna *et al.,* 2025). Additionally, a 2023 comparative study found that povidone-iodine exhibited faster and stronger efficacy against *MRSA* (including strain ATCC 43300), inhibiting biofilm formation at concentrations as low as 0.33% (Sienkiewicz *et al*., 2024).

A systematic review and meta-analysis in *Antimicrobial Agents and Chemotherapy* (2020) reported that povidone-iodine typically achieves bactericidal activity against MSSA and MRSA within 15–60 seconds. Complementing this, an orthopaedic irrigation protocol using 0.35% povidone-iodine has demonstrated significant reductions in surgical site infections when used as a 3-minute soak.

Again, this study found that Ciprofloxacin, the antibiotic control, showed strong inhibition against *S. aureus* (32.0 mm) and moderate inhibition against *P. aeruginosa* (14.0 mm), but was less effective against

 

*E. coli* (10.0 mm). The reduced activity against *E. coli* suggests possible emerging resistance. This aligns with global reports of increasing fluoroquinolone resistance among Enterobacteriaceae (Hooper and Jacoby, 2016). Its weaker inhibition of *E. coli* in this study supports previous findings from Adegoke *et al.* (2017) and Leaper *et al.* (2015) on emerging fluoroquinolone resistance**,** especially in Gram-negative organisms like *E. coli*.

Statistical analyses (ANOVA and Post Hoc tests) confirmed significant differences between treatments for all three organisms ($p < 0.05$). This indicates that antiseptic efficacy is not uniform across bacterial species and highlights the clinical importance of selecting appropriate agents for wound management.

## 4. Conclusion

This research demonstrated that antiseptics differ markedly in their efficacy against wound pathogens under in vitro conditions. Hydrogen peroxide emerged as the most effective broad-spectrum agent, while isopropyl alcohol exhibited organism-specific action, and povidone-iodine was largely ineffective at the tested concentration. These findings emphasize the need for evidence-based selection of antiseptics tailored to local microbial profiles rather than relying on routine, empirical use. The study provides region-specific data that can inform clinical decision-making in Nigeria and similar low-resource contexts, thereby advancing rational wound care, reducing the risk of treatment failure, and contributing to antimicrobial stewardship.

## Conflict of Interest

The authors declare no conflict of interest.

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