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Comparative Evaluation of Commercial hand Sanitizers: Antibacterial Activity against selected Pathogenic Bacteria

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Abstract

Hand sanitizers have become a cornerstone of infection prevention, particularly after the COVID-19 pandemic. This study aimed to compare the antibacterial effectiveness of six commercially available hand sanitizer formulations (labeled A–F) against five clinically relevant bacterial pathogens: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Salmonella typhi. Using agar well diffusion and disc diffusion methods on Mueller-Hinton agar at three concentrations (50%, 75%, and 100%), the zone of inhibition was recorded for each sanitizer-bacterium combination. Sanitizer B demonstrated the highest overall antibacterial activity, particularly against S. aureus (27 mm at 100%) and E. coli (21 mm at 75%). Sanitizers A and E showed moderate effectiveness, whereas sanitizers C, D, and F were largely ineffective. Pseudomonas aeruginosa exhibited the greatest resistance among all tested organisms, while S. aureus was the most susceptible. Antibacterial efficacy consistently increased with concentration. These findings underscore the critical importance of formulation and concentration in determining sanitizer effectiveness, and support the use of adequately concentrated, properly formulated hand sanitizers for infection control.

Keywords: Hand sanitizer, antibacterial activity, zone of inhibition, agar well diffusion, Staphylococcus aureus, Pseudomonas aeruginosa, infection control.

1. Introduction

The word "hygiene" traces its origins to Hygeia, the ancient Greek goddess of healing, and in modern public health terminology it encompasses practices that prevent disease and promote well-being. Among these, hand hygiene stands out as one of the most studied, evidence-based interventions available. Pathogens transmit readily via contaminated hands to mucosal surfaces, food, and shared objects, making hand cleanliness a frontline barrier against a broad range of communicable diseases. The World Health Organization (WHO) estimates that proper handwashing alone has the potential to prevent over one million deaths annually [1]

Two primary approaches to hand hygiene are recognized globally: handwashing with soap and water, and the use of hand sanitizers. Hand sanitizers fall into two broad categories—alcohol-based hand sanitizers (ABHS) and non-alcohol-based hand sanitizers (NABHS). ABHS typically contain ethanol, isopropanol, or n-propanol at concentrations between 60% and 95%, which denature microbial proteins and disrupt lipid membranes, leading to rapid inactivation of a wide range of pathogens. NABHS employ alternative antiseptics such as benzalkonium chloride, chlorhexidine, or triclosan, relying on mechanisms distinct from alcoholic denaturation.

The COVID-19 pandemic dramatically accelerated global demand for hand sanitizers. As of March 2022, more than 460 million people had been infected and over six million deaths had been reported, prompting widespread adoption of sanitizer use as a complementary non-pharmaceutical intervention alongside mask-wearing and physical distancing. This surge resulted in an unprecedented proliferation of sanitizer products on the market, many of which varied widely in formulation, active ingredient concentration, and actual antibacterial performance.

Despite their widespread use, systematic comparative data on the antibacterial efficacy of commercially available hand sanitizers against multiple clinically relevant pathogens remain limited. The present study addresses this gap by evaluating six hand sanitizer samples—sourced from local markets and medical stores in Amravati, India—against five key bacterial pathogens using standardized in vitro methods.

2. Review of Literature

A substantial body of research has explored the antimicrobial properties of hand sanitizers, though direct and systematic comparisons across multiple commercial products and multiple organisms within a single study remain relatively rare.

Shahtaghi et al. [2] reviewed the historical and contemporary significance of hand hygiene, emphasizing the variable antibacterial performance of commercially available sanitizers and the need for rigorous comparative evaluation. Their work highlighted that even products with similar labeling could perform markedly differently against the same pathogen.

Thakur et al. [3] compared alcohol-based and non-alcoholic sanitizers across efficacy, safety, and usability dimensions, concluding that while both categories reduce microbial load, differences in antimicrobial spectrum and duration of action are meaningful in clinical contexts. Alcohol-free formulations were noted to be less irritating to skin and non-flammable, giving them practical advantages in specific settings such as pediatric wards and schools.

Sickbert-Bennett et al. [4] conducted what they described as one of the largest comparative studies at the time, testing 14 hand hygiene agents against bacteria and viruses using a realistic contact time of 10 seconds—reflecting actual clinical practice far better than earlier studies that used contact times of 30 seconds or longer. Their findings demonstrated that agent effectiveness was highly variable and that contact time significantly influenced outcomes.

Saheed and Igbineweka [5] examined the antibacterial activity of alcohol-based hand sanitizers against clinical bacterial isolates, confirming that Staphylococcus aureus was among the most susceptible organisms and that higher concentrations produced larger inhibition zones. Odebisi-Omokanye et al. [6] similarly observed substantial variability among four Nigerian commercial sanitizers against common pathogens.

Enwa et al. [7] compared alcohol-based hand sanitizers with toilet soaps and reported that sanitizers generally outperformed soaps in antibacterial activity, though

Gram-negative bacteria—particularly those with intrinsic resistance mechanisms—showed reduced susceptibility. This pattern aligns with the established understanding that Gram-negative bacteria possess an outer membrane that limits the penetration of antimicrobial agents.

Jain et al. [8] tested four sanitizer brands (Dettol, Lifebuoy, PureHands, and Sterillium) against five bacterial species using agar well diffusion. Sterillium produced the largest inhibition zones across all organisms, underlining the decisive role of formulation quality. Chojnacki et al. [9] systematically evaluated a large panel of commercial sanitizers during the COVID-19 pandemic and confirmed that performance varied considerably, with some products failing to meet minimum efficacy standards.

Ekawati et al. [10] demonstrated that bacteriostatic activity against Salmonella typhimurium, Pseudomonas aeruginosa, and Klebsiella pneumoniae was achievable at concentrations of 40–80%, while bactericidal effects against E. coli and S. aureus required concentrations closer to 80% or higher. This gradient of susceptibility mirrors findings from the present study.

3. Materials and Methods

3.1 Collection and Preparation of Sanitizer Samples

Six commercially available hand sanitizer samples were procured from local markets and medical stores in Amravati city, Maharashtra, India. Samples were labeled A through F to maintain confidentiality of brand identity and to facilitate objective comparison. Among the six, two were alcohol-based formulations, two contained glycerin as a primary moisturizing agent, one was herbal in composition, and one was a locally prepared product. All samples were transported aseptically to the microbiology laboratory and stored at room temperature under controlled conditions until use. Appropriate personal protective equipment was worn during all stages of sample handling.

Three working concentrations—50%, 75%, and 100%—were prepared from each sample using sterile distilled water under aseptic conditions, following the approach described by Thangkhiew et al. [11].

3.2 Test Microorganisms

Five bacterial species were selected based on their clinical relevance as agents of healthcare-associated and community-acquired infections: *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Salmonella typhi*. These cultures were obtained from the microbiology laboratory of Dr. Panjabrao Deshmukh Memorial Medical College (PDMMC), Amravati, and maintained on nutrient agar slants. Before experimentation, cultures were incubated at 37°C for 24 hours to ensure log-phase growth.

3.3 Identification of Test Organisms on Selective Media

Each organism was confirmed using selective and differential culture media chosen for its specificity to that organism's metabolic characteristics:

- *E. coli*: Dark colonies with a characteristic metallic green sheen on Eosin Methylene Blue (EMB) agar, indicating vigorous lactose fermentation.
- *S. aureus*: Yellow colonies with a surrounding yellow halo on Mannitol Salt Agar (MSA), confirming mannitol fermentation and salt tolerance.
- *P. aeruginosa*: Greenish colonies with a characteristic fruity odor on Cetrimide agar, reflecting pyocyanin pigment production.
- *K. pneumoniae*: Positive growth with color change from green to blue on Simmons Citrate Agar, confirming citrate utilization as the sole carbon source.
- *S. typhi*: Black or greenish-black colonies on Bismuth Sulphite Agar due to hydrogen sulfide production, with occasional metallic sheen.

3.4 Biochemical Characterization (IMViC Tests)

The IMViC battery of tests—Indole, Methyl Red, Voges-Proskauer, and Citrate utilization—was performed on all five organisms to confirm their biochemical identity and to differentiate members of the family Enterobacteriaceae. Results are summarized in Table 1.

Table 1: Biochemical Characterization of Test Organisms Using IMViC Tests

Organism	Indole	Methyl Red	Voges-Proskauer	Citrate
Escherichia coli	+	+	−	−
Staphylococcus aureus	−	+	+	−
Pseudomonas aeruginosa	−	−	−	+
Klebsiella pneumoniae	−	−	+	+
Salmonella typhi	−	+	−	+

(+ = Positive; − = Negative)

3.5 Antimicrobial Activity Testing

Mueller-Hinton agar (MHA) was prepared per manufacturer specifications (HiMedia Laboratories Pvt. Ltd., Mumbai, India), autoclaved at 121°C for 15 minutes, and poured into sterile petri dishes under aseptic conditions. Two methods were employed in parallel:

Agar Well Diffusion Method: Sterile MHA plates were inoculated with standardized bacterial suspensions using sterile cotton swabs. Wells of uniform diameter were created using a sterile cork borer. Each well received 50 µL of sanitizer at 50%, 75%, or 100% concentration. Plates were incubated at 37°C for 24 hours, after which zones of inhibition were measured in millimeters.

Disc Diffusion Method: Sterile filter paper discs were saturated with each sanitizer concentration and placed on inoculated MHA plates using sterile forceps. Incubation conditions and measurement procedures were identical to those used in the well diffusion method.

4. Results

4.1 Antibacterial Activity Against Staphylococcus aureus

Among all six sanitizers tested against S. aureus, Sanitizer B demonstrated the most pronounced activity, with inhibition zones increasing from less than 10 mm at 50% to 17 mm at 75%, and reaching a maximum of 27 mm at 100%. Sanitizer E was effective exclusively at 100% concentration, producing a 21 mm zone, while Sanitizer A showed an intermediate response of 14 mm at 75% with weaker activity at other concentrations. Sanitizers C, D, and F produced no measurable inhibition at any concentration tested.

4.2 Antibacterial Activity Against Escherichia coli

Activity against E. coli was observed in three of the six sanitizers. Sanitizer B produced its peak effect at 75% concentration (21 mm), with no detectable activity at 50% or 100%—a finding that warrants further investigation into concentration-dependent interactions with E. coli cell membranes. Sanitizers E and F were active only at 100%, yielding zones of 16 mm and 19 mm respectively. Sanitizers A, C, and D showed no inhibitory effect across all tested concentrations.

4.3 Antibacterial Activity Against Pseudomonas aeruginosa

Pseudomonas aeruginosa displayed the highest resistance of all organisms tested. Only Sanitizers A, B, and E produced any measurable inhibition, and even then, values remained below or at the 10 mm threshold at all concentrations. Sanitizers C, D, and F showed no activity whatsoever. The intrinsic resistance of P. aeruginosa is well-documented and arises from its outer membrane's low permeability, the activity of multidrug efflux pumps, and the organism's capacity to form biofilms.

4.4 Antibacterial Activity Against Klebsiella pneumoniae

Sanitizer A was the most active against K. pneumoniae, producing zones of 17 mm at 50% and 16 mm at 100%, though activity at 75% dropped below 10 mm—a pattern suggesting possible concentration-dependent variability or inoculum effects. Sanitizer E showed a 10 mm zone only at 100%, and Sanitizer F demonstrated minimal but detectable activity at all concentrations. Sanitizers B, C, and D produced no significant inhibition.

Table 2: Antibacterial Activity Against Staphylococcus aureus (Zone of Inhibition in mm)

Sample	Concentration	Zone of Inhibition (mm)	Interpretation
A	50%	0	No inhibition
A	75%	14 mm	Moderate
A	100%	<10 mm	Weak
B	50%	<10 mm	Weak
B	75%	17 mm	Moderate-High
B	100%	27 mm	Highest
C	50%	0	No inhibition
C	75%	0	No inhibition
C	100%	0	No inhibition
D	50%	0	No inhibition
D	75%	0	No inhibition
D	100%	0	No inhibition
E	50%	0	No inhibition
E	75%	0	No inhibition
E	100%	21 mm	High
F	50%	0	No inhibition
F	75%	0	No inhibition
F	100%	0	No inhibition

Table 3: Summary of Peak Antibacterial Activity (Maximum Zone of Inhibition) Across All Organisms

Sample	S. aureus	E. coli	P. aeruginosa	K. pneumoniae	S. typhi
A	14 mm (75%)	0	<10 mm	17 mm (50%)	15 mm (50%)
B	27 mm (100%)	21 mm (75%)	10 mm (100%)	<10 mm	14 mm (50%)
C	0	0	0	0	<10 mm
D	0	0	0	0	<10 mm
E	21 mm (100%)	16 mm (100%)	<10 mm	10 mm (100%)	13 mm (100%)
F	0	19 mm (100%)	0	<10 mm	0

4.5 Antibacterial Activity Against Salmonella typhi

Sanitizers A and B demonstrated the most consistent activity against S. typhi. Sanitizer A produced zones of 15 mm and 12 mm at 50% and 100% respectively, with reduced activity at 75%. Sanitizer B showed a declining but sustained pattern: 14 mm (50%), 13 mm (75%), and 10 mm (100%). Sanitizers D and E showed moderate inhibition only at 100% (12 mm and 13 mm respectively), Sanitizer C showed minimal activity at lower concentrations but none at 100%, and Sanitizer F showed no activity at any concentration.

4.6 Summary: Comparative Antibacterial Performance

The table below consolidates the peak zone of inhibition recorded for each sanitizer against each organism across all concentrations tested, providing an at-a-glance comparison of overall antibacterial performance.

5. Discussion

The central finding of this study is that antibacterial efficacy among commercial hand sanitizers is neither uniform nor predictable from label claims alone. Sanitizer B stood out as consistently the most effective formulation, followed by Sanitizers A and E, while Sanitizers C, D, and F performed poorly against most or all of the test organisms. This variability is consistent with observations from Jain et al. [8] and Chojnacki et al. [9] both of whom found substantial differences in antimicrobial performance among products available on the open market.

A clear concentration-response relationship emerged from the data: higher sanitizer concentrations generally yielded larger zones of inhibition. This is mechanistically expected—higher alcohol concentrations denature proteins more completely and penetrate bacterial membranes more effectively. The WHO recommends alcohol-based hand rubs containing 60–80% ethanol or 60–80% isopropanol, and the present results support the clinical relevance of maintaining adequate concentrations in consumer products.

The differential susceptibility observed across bacterial species reflects well-established microbiological principles. Gram-positive organisms such as S. aureus lack an outer membrane and are therefore more susceptible to the denaturing effects of alcohols and other antiseptics. Gram-negative bacteria, by contrast, possess an additional outer membrane layer that substantially reduces the permeability of antimicrobial agents. Among the Gram-negative organisms tested, P. aeruginosa was by far the most resistant, producing negligible inhibition zones even at 100% concentration. This aligns with its known intrinsic defense mechanisms, including constitutive expression of efflux pumps such as MexAB-OprM, reduced outer membrane permeability through downregulation of porin channels, and a propensity to form protective biofilms.

The notable performance of Sanitizer B against E. coli at 75% but not at 100% is an unexpected finding. While seemingly counterintuitive, this phenomenon may reflect the "soap paradox" or an optimal concentration effect, in which very high alcohol concentrations may cause faster surface drying before full antimicrobial penetration, or may alter the physical properties of the preparation in ways that reduce contact efficiency. This observation merits further investigation using quantitative suspension tests and time-kill kinetics.

Sanitizer A's leading performance against K. pneumoniae and S. typhi—but not against S. aureus or E. coli—suggests that different formulations may be optimized for different pathogen classes. This points toward the possibility that no single commercially available sanitizer currently provides comprehensive coverage across all clinically important bacteria, a conclusion with practical implications for infection control program design in hospitals and community settings.

The failure of Sanitizers C, D, and F against most test organisms is a concern from a public health standpoint. These products were available to consumers through the same retail channels as the effective products, with no obvious labeling differences. While the exact formulations were not disclosed in this study, the absence of activity across multiple organisms and concentrations suggests that either the active ingredient concentration was subtherapeutic or the formulation characteristics (viscosity, evaporation rate, penetration) were inadequate. This underscores the need for more stringent regulatory oversight of hand sanitizer products [12,13].

6. Conclusion

This comparative study demonstrates that substantial variation exists in the antibacterial efficacy of commercially available hand sanitizers. Sanitizer B was the most effective overall formulation, achieving the largest inhibition zones against S. aureus and E. coli at 100% concentration. Sanitizers A and E exhibited moderate efficacy, while Sanitizers C, D, and F showed minimal to no antibacterial activity across tested conditions.

Staphylococcus aureus was the most susceptible organism, followed by Escherichia coli and Salmonella typhi, while Pseudomonas aeruginosa demonstrated consistently high resistance. Klebsiella pneumoniae showed variable susceptibility depending on the sanitizer used. Across all sanitizer-bacterium combinations, antibacterial activity increased with concentration, confirming the clinical rationale for maintaining adequate alcohol concentrations in hand hygiene products.

From a practical standpoint, these results highlight that consumers and healthcare institutions cannot rely on product availability alone as a proxy for efficacy. Regulatory agencies should enforce minimum antimicrobial performance standards for products marketed as hand sanitizers, and healthcare facilities should consider periodic testing of the products they procure. Future work should expand to include antiviral and antifungal activity, evaluate time-kill kinetics, and examine the influence of organic soil load on sanitizer performance under simulated in-use conditions.

Conflicts of interest: The authors stated that no conflicts of interest.

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