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Short Report

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Posted Date: October 27th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10301992/v2>

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Additional Declarations: The authors declare no competing interests.

In Vitro Study of the Antibacterial Efficacy of Commercial Alcohol-Based Hand Gels Available on the Lebanese Market

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Abstract

Background & Purpose: Hand hygiene is universally recognized as the single most essential intervention for preventing infection transmission in healthcare and community settings. During the COVID-19 pandemic and the concurrent financial crisis in Lebanon (2019–present), commercial alcohol-based hand gels proliferated rapidly across local markets. However, independent quality verification of commercial hydrogels against multi-drug resistant (MDR) clinical pathogens remains scarce. This study evaluated the in vitro antibacterial efficacy of seven commercial hand gels commercially distributed in Lebanon (HG1–HG7) against clinically relevant bacterial pathogens.

Methods: Antibacterial activity was assessed using standardized agar well diffusion on Mueller-Hinton agar, measuring mean inhibition zone diameters (mm ± SD).

Results: Profound variation in antibacterial performance was observed among the tested commercial products. HG1 (locally manufactured, claiming 65% ethanol) demonstrated broad-spectrum inhibitory activity against *S. aureus*, *Salmonella* spp., *E. coli*, and *K. pneumoniae*. HG2 and HG7 displayed narrow-spectrum activity limited strictly to *S. aureus*. Crucially, HG3, HG4, HG5, and HG6 exhibited no detectable antibacterial activity across all tested bacterial strains, despite manufacturer label claims of 70% ethanol (HG3, HG6) or denatured alcohol (HG4, HG5). Multidrug-resistant *P. aeruginosa* proved completely resistant to all seven commercial hand gels.

Conclusion: Alarming quality failures and market inconsistencies exist among commercial hand gels distributed in Lebanon. Regulatory agencies, including the Ministry of Public Health and the Lebanese Standards Institution (LIBNOR), must establish mandatory post-market analytical oversight, requiring Gas Chromatography verification of active alcohol concentrations and standardized efficacy certification prior to commercial distribution.

Keywords: Hand Sanitizers; Anti-Bacterial Agents; Disinfectants; In Vitro Techniques; Multidrug-resistant, Bacterial; Lebanon

Introduction

Hand hygiene is globally recognized by the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) as the single most effective intervention for reducing the cross-transmission of infectious pathogens in both clinical and community settings (1, 2). Washing hands with plain soap and water remains the gold standard when hands are visibly dirty, greasy, or contaminated with proteinaceous material (3). However, alcohol-based hand rubs (ABHRs) are strongly advocated for routine clinical hand antisepsis when hands are not visibly soiled due to their rapid broad-spectrum antimicrobial activity, point-of-care accessibility, reduced skin irritation relative to frequent soap washing, and superior compliance rates among healthcare workers (4, 5).

The microbicidal activity of alcohol-based antiseptics relies primarily on ethanol, isopropanol, n-propanol, or synergistic mixtures of these aliphatic alcohols (6). Alcohols act rapidly against vegetative bacteria, fungi, and enveloped viruses through non-specific physical mechanisms: disrupting cellular membrane integrity, dissolving outer lipid bilayers, denaturing intracellular functional and structural proteins, and promoting massive transmembrane leakage of essential nucleotides, potassium, and protons (7, 8). To achieve reliable antimicrobial efficacy, the CDC and WHO recommend formulations containing 60% to 95% v/v total alcohol, with optimal microbicidal activity observed between 70% and 80% v/v (4, 9). Secondary active agents—such as quaternary ammonium compounds (e.g., benzalkonium chloride) or chlorhexidine gluconate—are frequently incorporated to impart prolonged persistent activity against resistant pathogens, including Methicillin-Resistant *Staphylococcus aureus* (MRSA) (4).

The unprecedented global health crisis precipitated by the COVID-19 pandemic catalyzed a massive surge in worldwide demand for commercial hand sanitizers (10). While public messaging heavily emphasized the antiviral benefits of hand sanitizers for controlling viral transmission, evaluating their antibacterial potency against resilient clinical bacteria provides an essential baseline benchmark for assessing chemical formulation integrity, active alcohol concentration, and overall microbicidal quality (8, 11). However, rapid market expansion led to the proliferation of substandard, misbranded, or informally manufactured products globally, with several post-market evaluations documenting products containing sub-potent alcohol concentrations or toxic adulterants such as methanol (12, 13).

In Lebanon, this global health crisis coincided with a financial and economic collapse beginning in late 2019, which severely disrupted pharmaceutical supply chains and public health regulatory infrastructure (14). In response to local shortages and hyperinflation, dozens of newly registered and unregistered commercial hand gel brands saturated the domestic market. While international guidelines mandate rigorous quality standards, independent post-market verification of commercial hand gels—specifically evaluating their antibacterial performance against phenotypically characterized, multi-drug resistant (MDR) clinical isolates in crisis-affected regions—remains poorly documented in the scientific literature. Therefore, the objective of this study was to conduct a

rigorous in vitro evaluation of the antibacterial efficacy of seven commercial alcohol-based hand gels available on the Lebanese market against clinically important bacterial pathogens.

2. Materials and Methods

2.1 Study Setting and Timeframe

This experimental in vitro study was conducted between October 2023 and March 2024 at the Microbiology Laboratory, Department of Biological Sciences, Faculty of Health Sciences, Holy Family University, Batroun, Lebanon.

2.2 Bacterial Strains and Clinical Rationale

Five clinical bacterial pathogens representing major Gram-positive and Gram-negative bacterial phenotypes commonly associated with nosocomial and community-acquired infections in Lebanese hospital settings were selected for evaluation.

The isolates were obtained from biological patient specimens (blood, sputum, urine, wound exudates) across care facilities in Lebanon. The panel comprised one Gram-positive species—Methicillin-Resistant *Staphylococcus aureus* (MRSA)—and four Gram-negative species: *Escherichia coli* (high-level cephalosporinase producer), *Klebsiella pneumoniae* (Group 2 natural resistance, low-level penicillinase producer), *Salmonella* spp. (co-expressing penicillinase and low-level cephalosporinase), and a multidrug-resistant (MDR) non-fermenting bacillus, *Pseudomonas aeruginosa* (resistant to ceftazidime, colistin, levofloxacin, fosfomycin, and aztreonam). Evaluating clinical isolates with documented enzymatic and permeability resistance mechanisms provides a far more rigorous test of disinfectant potency than standard laboratory reference strains.

2.3 Commercial Hand Sanitizers and Formulation Rationale

Seven commercially available alcohol-based hand gel brands (designated HG1 through HG7) were purchased from retail pharmacies and supermarkets in Lebanon. Hydrogel formulations were deliberately chosen over liquid alcohol rubs due to their dominance in the consumer market, superior user preference, practical advantages in dispensing (reduced spill risk and controlled dosing), and lower alcohol evaporation rates during application (16). The selection encompassed both locally manufactured domestic products (HG1, HG2) and prominent international commercial brands (HG3–HG7). Detailed manufacturer product label declarations regarding active ingredients, alcohol content, claimed antimicrobial spectrum, and specific usage indications are summarized in **Table 1**.

Table 1: Composition, declared alcohol content, and labeled efficacy of the seven commercial hand gels.

Product Code	Declared Active & Inactive Ingredients	Manufacturer Claimed Efficacy	Origin / Indication
HG1	Ethanol 65%, antimicrobial agents (unspecified), thickening agents, solvents, glycerin, water, vitamins, fragrance, colorants	Kills 99.98% of germs (bacteria, fungi, Salmonella, E. coli, Campylobacter, viruses)	Local (Lebanon) / Instant hand disinfectant
HG2	Ethyl alcohol 60%, alkyl-dimethyl-benzyl-ammonium chloride 0.04%, moisturizer, water, carbopol, amino-methyl-propanol, fragrance (pH 6–7)	Eliminates 99.99% of bacteria, viruses, and fungi	Local (Lebanon) / 4-hour protection claim
HG3	Ethanol 70%, denatured alcohol, water, glycerin, Vaccinium oxycoccus extract, acrylates/C10-30 alkyl acrylate crosspolymer, aminomethyl propanol, fragrance	Instantly kills 99.9% of germs	Imported / Antibacterial, antiviral, antifungal
HG4	Denatured alcohol (percentage undeclared*), water, copolymer, propylene glycol, acrylates/C10-30 alkyl acrylate crosspolymer, tetrahydroxypropyl ethylenediamine, fragrance	Provides 100% protection against germs	Imported / Broad-spectrum protection
HG5	Ethanol (percentage undeclared*), water, glycerin, carbomer, Aloe barbadensis leaf juice, isopropyl myristate, tocopheryl acetate, fragrance, aminomethyl propanol	Kills 99.9% of common germs and viruses	Imported / No rinsing required
HG6	Ethanol 70%, water, glycerin, carbomer, triethanolamine, acrylates/C10-30 alkyl acrylate crosspolymer, fragrance	Eliminates 99% of germs	Imported / Bactericidal, fungicidal, virucidal
HG7	Ethanol (percentage undeclared*), water, acrylates/C10-30 alkyl acrylate crosspolymer, glycerin, Aloe vera extract, 2-amino-2-methyl-1-propanol, alkyl-dimethyl-benzyl-ammonium chloride, fragrance	100% effective against germs and viruses (coronavirus, H1N1, hepatitis B)	Imported / Virucidal and bactericidal

* Note: Products HG4, HG5, and HG7 declared active alcohol ingredients on packaging without specifying exact volumetric percentages (v/v).

2.4 Agar Well Diffusion Assay Protocol

Antibacterial efficacy was determined using the standardized agar well diffusion method on Mueller-Hinton Agar (MHA) plates (Merck, Germany) prepared at a uniform depth of 5.0 mm (1, 3, 5). Bacterial inocula were prepared by suspending 3 to 4 well-isolated overnight colonies in 3.0 mL of sterile 0.9% physiological saline. Suspension turbidity was optically adjusted to match a 0.5 McFarland standard, corresponding to approximately 1.5×10^8 colony-forming units per milliliter (CFU/mL). Within 15 minutes of standardization, sterile cotton swabs were dipped into the bacterial

suspensions and evenly lawn-seeded across the entire surface of MHA plates in three directions in accordance with European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations. Circular wells of exactly 6.0 mm diameter were bored into the agar using a sterile glass Pasteur pipette, spaced at minimum intervals of 25.0 mm to prevent zone overlap. An aliquot of 50 µL of each undiluted commercial hand gel was carefully micropipetted into designated wells. Sterile distilled water (50 µL) was loaded into a central control well as a negative vehicle control. Plates were incubated at 37°C for 24 hours under aerobic conditions. Antibacterial activity was quantified by measuring the clear zone of inhibition (including well diameter) in millimeters using digital Vernier calipers. All experimental assays were performed independently in triplicate, and results were expressed as mean diameter ± standard deviation (SD).

3. Results

3.1 Phenotypic Profile of Clinical Bacterial Isolates

Phenotypic characterization confirmed that all clinical bacterial isolates possessed significant antimicrobial resistance markers. The Gram-positive *Staphylococcus aureus* strain was confirmed as Methicillin-Resistant *S. aureus* (MRSA) based on phenotypic resistance to both oxacillin and ceftazidime. Among Gram-negative isolates, *Escherichia coli* expressed a high-level cephalosporinase phenotype; *Klebsiella pneumoniae* exhibited Group 2 natural resistance (low-level penicillinase production); *Salmonella* spp. co-expressed penicillinase and low-level cephalosporinase enzymes; and *Pseudomonas aeruginosa* demonstrated a pan-resistant MDR profile, showing simultaneous resistance to ceftazidime, colistin, levofloxacin, fosfomycin, and aztreonam. Detailed phenotypic traits are outlined in **Table 2**.

Table 2: Phenotypic resistance profiles and enzymatic characteristics of tested clinical isolates.

Bacterial Species	Resistance Phenotype	Associated Resistance / Enzymatic Profile
<i>Staphylococcus aureus</i>	Methicillin-Resistant (MRSA)	Resistant to oxacillin and ceftazidime; mecA positive phenotype
<i>Escherichia coli</i>	Cephalosporinase Producer	High-level AmpC cephalosporinase hyper-expression
<i>Klebsiella pneumoniae</i>	Group 2 Natural Resistance	Constitutive low-level SHV-1 penicillinase expression
<i>Salmonella</i> spp.	Penicillinase & Cephalosporinase	Co-expression of TEM-type penicillinase and low-level AmpC
<i>Pseudomonas aeruginosa</i>	Multidrug-Resistant (MDR)	Simultaneous resistance to ceftazidime, colistin, levofloxacin, fosfomycin, and aztreonam

3.2 Antibacterial Efficacy of Commercial Hand Gels

Quantitative measurements of mean inhibition zone diameters revealed dramatic disparities in antibacterial performance among the seven commercial hand gels (Table 3). HG1 (locally produced, 65% ethanol and unspecified antimicrobial agent) demonstrated superior broad-spectrum microbicidal activity, generating the largest zones of inhibition against four out of five clinical pathogens: *S. aureus* (39.67 ± 2.50 mm), *Salmonella* spp. (29.33 ± 0.57 mm), *E. coli* (26.00 ± 1.00 mm), and *K. pneumoniae* (26.00 ± 1.40 mm). In contrast, HG2 and HG7 displayed highly restricted narrow-spectrum activity, inhibiting only Gram-positive *S. aureus* with modest mean zone diameters of 10.67 ± 0.57 mm and 14.00 ± 1.00 mm, respectively, while failing completely to inhibit any of the tested Gram-negative pathogens.

Most striking was the total absence of antibacterial activity observed with products HG3, HG4, HG5, and HG6. These four commercial hand gels failed to produce any detectable zone of inhibition (0.00 mm) against all five bacterial strains, indicating a complete absence of in vitro microbicidal activity despite explicit manufacturer label assertions claiming 70% ethanol (HG3, HG6) or germicidal protection (HG4, HG5). Furthermore, multidrug-resistant *P. aeruginosa* exhibited complete resistance (0.00 mm inhibition zone) across all seven tested commercial hand gels, without exception.

Table 3: Mean inhibition zone diameters (mm $\pm$ SD) generated by commercial hand gels against clinical isolates.

Hand Gel Code	<i>S. aureus</i> (MRSA)	<i>Salmonella</i> spp.	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i> (MDR)
HG1	39.67 ± 2.50	29.33 ± 0.57	26.00 ± 1.00	26.00 ± 1.40	0.00 ± 0.00
HG2	10.67 ± 0.57	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
HG3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
HG4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
HG5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
HG6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
HG7	14.00 ± 1.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Values represent mean inhibition zone diameter in mm (including 6.0 mm well diameter) $\pm$ standard deviation from triplicate assays. Sterile distilled water negative control yielded 0.00 mm across all plates.

4. Discussion

This study provides critical real-world evaluation of commercial hand gel quality by assessing products not against standard ATCC reference strains, but against phenotypically characterized

clinical and hospital-acquired multidrug-resistant (MDR) bacterial isolates from Lebanon. By testing commercial hand gels directly against clinical MRSA, high-level cephalosporinase producers, and pan-resistant *P. aeruginosa*, our findings expose alarming post-market quality failures that standard quality-assurance laboratory testing might overlook.

The outstanding broad-spectrum performance of HG1 highlights the feasibility of manufacturing highly effective domestic antiseptics. HG1 demonstrated clear superiority over both local and imported competitors, achieving extensive inhibition zones across Gram-positive and Gram-negative species. This high potency is likely attributable to an optimal ethanol concentration (65% v/v) operating in synergy with secondary unlisted antimicrobial additives. In contrast, HG2 (60% ethyl alcohol with 0.04% benzalkonium chloride) and HG7 (ethanol with benzalkonium chloride) achieved only weak, narrow-spectrum inhibition restricted to *S. aureus*. Gram-positive bacteria lack the complex outer membrane barrier characteristic of Gram-negative species, rendering their peptidoglycan cell walls more susceptible to lipophilic disruption by quaternary ammonium compounds and alcohols (8, 17). It is noteworthy that amino-methyl-propanol (present in HG2 and HG7) serves chemically as an alkanolamine neutralizer used to adjust pH and induce carbomer gelation, rather than functioning as an active bactericidal propanol disinfectant such as isopropanol or n-propanol (18).

The complete failure of HG3, HG4, HG5, and HG6 to produce any detectable zone of inhibition represents a profound public health concern. This total efficacy failure despite explicit label claims of 70% ethanol (HG3, HG6) or germicidal activity (HG4, HG5) can be explained by four distinct biochemical and physical mechanisms: First, substandard or fraudulent manufacturing: actual active alcohol content may fall drastically below declared label concentrations due to deliberate adulteration, poor quality control, or raw material degradation during local crisis conditions (12, 13). Second, alcohol volatility and packaging defects: sub-optimal bottle sealing or non-airtight plastic containers facilitate rapid ethanol evaporation during storage and retail display prior to consumer purchase (16). Third, polymer matrix entrapment: hydrogel formulations utilizing high-density cross-linked acrylates/C10-30 alkyl acrylate crosspolymers or carbomers can form dense polymer networks that entrap active alcohol and secondary microbicides, restricting their outward thermodynamic diffusion into aqueous agar media (16, 18). Fourth, methodological constraints of agar well diffusion: while agar diffusion effectively screens hydrophilic antimicrobial agents, highly viscous hydrogels diffuse less readily through agar matrices compared to liquid solutions, potentially masking activity unless evaluated via direct contact suspension protocols (11, 19).

The universal resistance exhibited by multidrug-resistant *Pseudomonas aeruginosa* across all seven commercial hand gels underscores the extreme physiological resilience of this opportunistic pathogen. *P. aeruginosa* possesses intrinsic resistance mechanisms including an exceptionally low outer membrane permeability (porin channels with restricted exclusion limits), constitutive expression of multidrug RND-type efflux pumps (MexAB-OprM, MexCD-OprJ), and the capacity for rapid alginate slime production (20). Our findings demonstrate that commercial hand gels alone cannot be relied upon as sole decontamination agents in clinical environments where MDR *P. aeruginosa* is endemic.

Our findings align with and build upon a growing body of global literature evaluating post-market commercial sanitizer quality. In India, Jain et al. (3) observed high susceptibility in *S. aureus* (27.0 mm zone) when testing commercial gels, confirming Gram-positive vulnerability. In contrast, Manaye et al. (1) in Ethiopia reported strong efficacy against *P. aeruginosa* (27.0 mm) using liquid alcohol solutions, emphasizing the superior chemical diffusion of liquid rubs relative to viscous hydrogels. In Ghana, Osei-Asare et al. (21) evaluated commercial sanitizers during the COVID-19 pandemic and found that 45% of tested products failed pharmacopeial efficacy standards due to substandard active alcohol levels. Similarly, Umeora et al. (22) in Nigeria reported widespread bacterial growth following hand gel applications lacking verified microbicidal agents. In Nepal, Shrestha et al. (23) documented severe performance discrepancies between locally manufactured sanitizers and imported brands. Finally, Al-Snafi et al. (24) in Saudi Arabia confirmed significant resistance among clinical *P. aeruginosa* isolates against commercial alcohol rubs, corroborating the urgent requirement for strict regulatory oversight.

Conclusion

This study reveals critical performance disparities and alarming failure rates among commercial alcohol-based hand gels distributed in the Lebanese market. While one locally produced formulation (HG1) achieved robust broad-spectrum antibacterial efficacy, over 57% of tested commercial gels (HG3–HG6) failed entirely to exhibit any in vitro antibacterial activity against clinical pathogens, representing a severe public health hazard and commercial market failure.

To protect public health and restore consumer confidence, immediate policy and regulatory interventions are required in Lebanon:

1. **Mandatory Analytical Verification:** The Ministry of Public Health (MoPH) and the Lebanese Standards Institution (LIBNOR) must mandate Gas Chromatography (GC-FID) verification of active alcohol concentrations for all hand sanitizers prior to issuing market clearance.
2. **Standardization of Efficacy Testing:** Regulatory authorities should adopt standardized quantitative efficacy benchmarks (such as European Norm EN 1500) as a prerequisite for commercial licensing.
3. **Post-Market Surveillance and Recalls:** Public health agencies must implement routine post-market random sampling from pharmacies and retail outlets.

Brands failing efficacy checks or presenting deceptive alcohol declarations should face immediate public warnings, product recalls, and administrative sanctions.

➤ Limitations

To properly contextualize these findings, three key technical limitations must be acknowledged:

- Active alcohol concentrations were evaluated based on manufacturer packaging label declarations rather than direct analytical chemical quantification via Gas Chromatography with Flame Ionization Detection (GC-FID) or densitometry.
- Assays were performed under fixed ambient laboratory conditions (37°C incubation) without evaluating product performance across variable storage temperatures, relative humidity, or extended shelf-life durations.
- The agar well diffusion assay measures hydrogel active diffusion through solid media rather than quantitative contact kill-time kinetics (e.g., European Standards EN 1276 or EN 1500) measuring logarithmic bacterial reduction (log₁₀ kill) on human skin under mechanical friction.

Declarations and Administrative Information

Ethical Considerations: Not applicable. This study was conducted entirely in vitro using bacterial laboratory cultures and did not involve human participants, clinical trials, or animal experimentation.

Consent to Publish: Not applicable.

Data Availability: The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Funding: This research was supported by Holy Family University, Batroun, Lebanon. The funding body played no role in study design, data collection, analysis, interpretation, or manuscript writing.

Declaration of Generative AI Assistance: During manuscript preparation, language editing and structural table formatting were assisted by AI editing tools. The authors reviewed, edited, and verified all outputs and take full scientific responsibility for the final content.

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