



2023

Evaluation of glycerol antiseptic effect on Gram positive and Gram negative bacteria

Asmaa S. Sleem

Medical microbiology and immunology Department, Faculty of Medicine, Menoufia University, Shibinelkom, Menoufia, Egypt., sasmaashaaban@yahoo.com

Samar S. Salman

Clinical pathology Department, Faculty of Medicine, Menoufia University, Shibinelkom, Menoufia, Egypt

Wafaa A. Shehata

Dermatology, Andrology & STDs Department, Faculty of Medicine, Menoufia University, Shibinelkom, Menoufia, Egypt

Amal M. Dawoud

Medical microbiology and immunology Department, Faculty of Medicine, Menoufia University, Shibinelkom, Menoufia, Egypt

Follow this and additional works at: <https://www.menoufia-med-j.com/journal>



Part of the [Medicine and Health Sciences Commons](#)

Recommended Citation

Sleem, Asmaa S.; Salman, Samar S.; Shehata, Wafaa A.; and Dawoud, Amal M. (2023) "Evaluation of glycerol antiseptic effect on Gram positive and Gram negative bacteria," *Menoufia Medical Journal*: Vol. 36: Iss. 3, Article 9.

DOI: <https://doi.org/10.59204/2314-6788.1100>

This Original Study is brought to you for free and open access by Menoufia Medical Journal. It has been accepted for inclusion in Menoufia Medical Journal by an authorized editor of Menoufia Medical Journal. For more information, please contact menoufiamedicaljournal@yahoo.com.

ORIGINAL STUDY

Evaluation of Glycerol Antiseptic Effect on Gram Positive and Gram Negative Bacteria

Asmaa S. Sleem ^{a,*}, Samar S. Salman ^b, Wafaa A. Shehata ^c, Amal M. Dawoud ^a

^a Medical Microbiology and Immunology Department, Faculty of Medicine, Menoufia University, Shibinelkom, Menoufia, Egypt

^b Clinical Pathology Department, Faculty of Medicine, Menoufia University, Shibinelkom, Menoufia, Egypt

^c Dermatology, Andrology & STDs Department, Faculty of Medicine, Menoufia University, Shibinelkom, Menoufia, Egypt

Abstract

Objectives: To assess glycerol antibacterial activity against both Gram positive and negative microorganisms, detect impact of increasing glycerol concentration in bacterial cultures on extra/intracellular Glycerol-3-phosphate (G3P) concentration, evaluate antiseptic efficacy of different glycerol/alcohol formulations and monitor HCWs tolerability to these formulations to recommend the best antiseptic preparation *in vitro*.

Background: The progressively rising nosocomial infections have enforced the need for novel infection prevention modalities. Glycerol emerges as a significant and cheap antibacterial agent against both Gram positive and negative organisms without any toxic effects.

Methodology: Different glycerol/ethanol formulations were evaluated for both bacterial inhibitory and bactericidal activity against selected bacteria (4 reference and 5 clinical strains). Effect of glycerol/ethanol combination was tested by checkerboard method. Colorimetric assay of extra and intracellular bacterial concentration of G3P was done. Skin tolerance of involved HCWs was evaluated against obtained bactericidal formulations including; 80% ethanol (E80) without glycerol (G0) as group (A), E80G1.45 (B), E80G20 (C) and E0G80 as group (D).

Results: Microbial growth inhibition was detected at G40E40, G40E60, G1.45 E80 and G20 E80 for most strains. No visible growth (bactericidal effect) was detected with G80, G20 E80 and G1.45 E80. Glycerol/ethanol combination showed synergistic effect against *Enterococcus faecalis* and biofilm-producing MRSA with an additive effect against *Proteus mirabilis*, *staphylococcus aureus*. *Acinetobacter baumannii* displayed indifference. No antagonism was detected. G3P levels were positively correlated with added glycerol concentration. E80G20 achieved HCWs maximum tolerability (100%) followed by E0G80 (92.3%).

Conclusion: E80G20 and E0G80 were superior to WHO recommended E80G1.45. However, more advanced studies are needed.

Keywords: Bactericidal, Checkerboard, Glycerol, Hand rub, Tolerability

1. Introduction

Hospital acquired bacterial infections have been increasing recently, with the lack of available therapeutic options. To combat globally increased morbidity and mortality, novel infection prevention and control modalities are needed [1]. Glycerol emerges as a significant worth organic compound that is mostly applied in cosmetics for its skin protection and antibacterial effects. It can be obtained from plant and animal sources without any

environmental harm (green compound) being non-toxic, non-expensive and renewable [2].

Glycerin is Food and Drug Administration-approved antimicrobial agent for infected wounds. Many reports detected that 85% solution of glycerin shows anti-inflammatory and bacterial toxic effects against both Gram positive and Gram negative organisms [3]. Glycerol antibacterial effect occurs through Glycerol-3-Phosphate (G3P) metabolic products that induce oxidative stress, DNA damage and gene expression changes. During aerobic

Received 13 June 2023; revised 16 August 2023; accepted 27 August 2023.
Available online 11 October 2023

* Corresponding author at: Faculty of Medicine, Menoufia University, Egypt.
E-mail address: sasmaashaaban@yahoo.com (A.S. Sleem).

<https://doi.org/10.59204/2314-6788.1100>

2314-6788/© 2023 The Authors. Published by Menoufia University. This is an open access article under the CC BY-NC-SA 4.0 license (<https://creativecommons.org/licenses/by-nc-sa/4.0/>).

microbial metabolism, glycerol is phosphorylated to G3P prior to entering the gluconeogenic or glycolytic pathway. However, the production of large amount of G3P is toxic to bacteria [4].

The WHO developed alcohol-based hand rub (ABHR) contains ethanol 80% with glycerol 1.45% to avoid skin irritation. The occurrence of dermatitis among hospital personnel has bad impacts on hand hygiene compliance [5]. ABHR is generally available in rich countries with low availability in poor countries due to its high cost. Several studies have shown glycerol enhancing the antimicrobial efficacy of the alcohol while, others have shown antagonistic combination effect. The best formulation of glycerol as hand antiseptic agent is still unclear [6].

Here, we are trying to assess glycerol antibacterial activity against both Gram positive and Gram negative strains and detect impact of increasing glycerol concentration added to the bacterial cultures on extra- and intracellular concentration of G3P. Also we are aiming to evaluate antiseptic efficacy of different glycerol/alcohol formulations and monitor health care workers (HCWs) skin tolerability to these formulations to recommend the best antiseptic preparation *in vitro*.

2. Materials and methods

2.1. Bacterial reference strains purification

Four reference strains *Acinetobacter baumannii* (ATCC CAP 2022), *Proteus mirabilis* (ATCC 14153), Methicillin-resistant *Staphylococcus aureus* (ATCC 43300) and *Enterococcus faecalis* (ATCC 29212) were used in this study. Reference strains were obtained from Medical Microbiology section, Clinical Pathology laboratory, Faculty of Medicine, Menoufia University. During the study, all microorganisms were previously cultivated on Mannitol, MacConkey and human blood agar to confirm their purity at 37 °C for 48 h prior to testing.

2.2. Bacterial clinical isolates identification

Different clinical specimens were immediately delivered to Medical Microbiology laboratory to be processed and examined. Each sample was inoculated on blood agar, MacConkey and Mannitol-salt agar (Oxoid LLC, UK) then incubated aerobically at 37 °C for 24–48 h. Full identification of obtained colonies was completed up to species level via conventional techniques according to the standard microbiological methods [7] then confirmed by Vitek 2 identification system (bioMérieux, France). The clinical isolates in current study included five

multidrug resistant isolates; *A. baumannii* and *P. mirabilis* as Gram negative bacterial samples, *E. faecalis*, nonbiofilm producing methicillin-resistant *Staph. aureus* (MRSA) and biofilm producing MRSA as Gram positive bacteria. All were isolated from hospitalized patients after 48 h of admission.

Evaluation of the antibacterial activity of different formulations of glycerol with and without ethanol.

Selected bacterial strains (4 reference and 5 clinical isolates) were evaluated for their sensitivity to different glycerol formulations; using sterile distilled water, 100% ethanol and pure glycerol (99.9%) represented in three categories (i) 20%, 40%, 60%, 80% and 99.9% pure glycerol as G20, G40, G60, G80 and G99.9% respectively (ii) 20%, 40%, 60% and 80% ethanol (WHO recommended bactericidal concentration) (iii) mixed glycerol (G) and ethanol (E) as follow; Glycerol 20% Ethanol 20% (G20 E20), G20 E40, G20 E60, G20 E80, G40 E20, G40E40, G40.

E60, G60E20, G60 E40, G80 E20 and finally G1.45 E80 which is WHO recommended alcohol-based hand rub formula.

2.3. Broth micro-dilution assay of bacterial inhibitory concentration

Broth micro-dilution assay was performed on sterile 96-well micro-titre plate (Catalog number: KG10096, Kirgen, Korea). Different formulations of glycerol with and without ethanol were prepared in sterile distilled water as stock. A total of 100 µl of different mixtures was added to each well of used micro-titer plate.

Bacterial inoculum was prepared according to CLSI guidelines [8]; making Muller Hinton broth (MHB) bacterial suspension with adjusted turbidity equivalent to 0.5 McFarland standard followed by 100 times dilution. Now, each 100 µl inoculation contained approximate range of (2–5) ×10⁵ CFU/ml. After that, 100 µl of bacterial suspension was transferred into each well of the micro-titer plate reaching a final volume of 200 µl/well followed by 24 h incubation at 37 °C. Three wells were inoculated for each concentration per each bacterial species. For each strain, 100 µl of bacterial inoculum mixed with 100 µl sterile water were used as positive growth control while, 100 µl non-inoculated MHB mixed with 100 µl sterile water was negative growth control [9]. Bacterial growth at each formulation was measured in triplicate at 620 nm using Infinite (Tecan, Korea) followed by standard deviation calculation to be compared with positive and negative control readings.

To assess the bactericidal glycerol formulations; Ten µl from each well of the micro-titre plate that showed visible, and no visible growth along with the

control were further spotted on BHI agar and incubated at 37 °C for 24 h and were observed for any bacterial growth. Absent growth indicated bactericidal effect [8].

2.4. Testing efficacy of glycerol ethanol combination

To check the effect of the association of glycerol with ethanol, we used the checkerboard method. Serial double dilution of glycerol (80%, 40%, 20%, 10%, 5%, 2.5%, 1.45% and zero%) were combined with serial double dilution of ethanol (80%, 40%, 20%, 10%, 5%, 2.5%, 1.45% and zero %) and then microbial inoculum of each strain were inoculated independently into each well of a 96-well microtiter plate. All plates were incubated at 37 °C for 18–24 h under aerobic conditions. After that, wells with turbidity were indicated to have bacterial growth. Then the MICs for the individual glycerol and ethanol in the checkerboard method were recorded. The lowest fractional inhibitory concentration index (FICI) was used to define synergy and analyze data from the checkerboard assay.

FIC for substance=(MIC of substance in combination)/(MIC of substance alone). Finally, $FICI = FIC \text{ for glycerol} + FIC \text{ for ethanol}$. $FICI \leq 0.5$ means synergism, $0.5 < FICI < 1$ indicates addition, $1 < FICI < 4$ equals indifference, while $FICI \geq 4$ refers to antagonism [10].

2.5. Glycerol-3-phosphate (G3P) colorimetric assay

We used Glycerol-3-Phosphate G3P Colorimetric Assay Kit (Catalog #K641- 100-Sigma Aldrich). The kit included G3P assay buffer, G3P enzyme mix, G3P probe and G3P standard. The culture supernatant was used for G3P measurement. For intracellular G3P assays, the bacterial cells were centrifuged then lysed in G3P assay buffer followed by second centrifugation for 5 min G3P amount was evaluated according to the standard curve and manufacturer instructions [11].

2.6. Hand hygiene and tolerability assay

We conducted a random double-blind study on 39 HCWs from November, 2022 to February, 2023. All participants were invited and agreed to share in this work. The study was approved by the Research Ethics Committee.

All formulations were prepared in sterile containers under aseptic conditions. Participants could wash their hands with soap and water. They could wear powder-free gloves. Used formulations

contained 80% ethanol with the addition of glycerol in variable concentrations; 0% (group A), 1.45% (group B), 20% (group C), while last group (group D) contain 80% glycerol without ethanol. These different formulations were chosen based on the formerly tested bactericidal activity.

Skin tolerance evaluation (redness, itching, dryness) of involved participants was obtained after one week of continuous use for each formulation.

2.7. Statistical analysis

Data coding, validation and analysis were conducted by the Statistical Package for the Social Sciences (SPSS), version 20 (SPSS Inc., Chicago, IL, USA). Continuous variables are expressed as mean and SD. Categorical variables are expressed as frequencies and percent. Chi square, ANOVA and Spearman's correlation tests were used. A significance level of $P < 0.05$ was used in all tests.

3. Results

There were high statistically significant variations between bacterial growth inhibition with different glycerol formulations among clinical and reference strains. Regarding bacterial inhibitory activity of glycerol, retarded microbial growth was detected at G40E40, G40E60, G1.45 E80 and G20 E80 for most strains (Table 1).

G20 E80, G40E60, G60E20 and G1.45 E80 formulations had the highest bacterial inhibitory effects on *E. faecalis*, E20G20, G20 E80, G40E60, G40E80 and G1.45 E80 for *A. baumannii* and G20 E60, G40E40, G40E60 for MRSA especially nonbiofilm producers. Prepared formulations with 20% and 40% glycerol combinations showed enhanced inhibitory effects with increasing ethanol concentrations as compared to positive and negative readings.

Regarding *P. mirabilis*, different glycerol 40% mixed ethanol combinations showed antibacterial effects. On the other hand, biofilm forming MRSA clinical strains were only inhibited by E80 containing formulations with high resistance profile, while nonbiofilm producing clinical MRSA was susceptible to G80, G40E40 and G40E60 formulations.

After all the wells were sub-cultured on Brain heart infusion agar, no visible growth was detected from concentrations G80, G20 E80 and G1.45 E80 with dominant bactericidal effect. G40 E60 was the second effective formulation against tested strains except biofilm producing MRSA. Biofilm producing MRSA were not affected by most concentrations similar to *E. faecalis*. On the other hand, *P. mirabilis* was killed by all formulations except G20 (Table 2).

Table 1. Assessment of bacterial growth with different formulations of glycerol on reference ATCC strains & clinical isolates.

ATCC strains	<i>Enterococcus faecalis</i>	<i>Acinetobacter baumannii</i>	<i>Proteus mirabilis</i>	<i>Staphylococcus aureus</i>	
G20	0.0885 ± 0.00941	0.0897 ± 0.00774	0.1011 ± 0.00964	0.0955 ± 0.00831	
G20 E20	0.0852 ± 0.00803	0.0780 ± 0.06501	0.1007 ± 0.01166	0.0897 ± 0.00826	
G20 E40	0.0864 ± 0.00843	0.0721 ± 0.00600	0.0899 ± 0.00906	0.0896 ± 0.00843	
G20 E60	0.0710 ± 0.00472	0.0718 ± 0.00554	0.1100 ± 0.01039	0.0686 ± 0.00479	
G20 E80	0.0687 ± 0.00370	0.0698 ± 0.00491	0.0801 ± 0.00774	0.0706 ± 0.00502	
G40	0.0925 ± 0.00958	0.0900 ± 0.00831	0.0988 ± 0.01045	0.0968 ± 0.00878	
G40 E20	0.0735 ± 0.00745	0.0721 ± 0.00606	0.0751 ± 0.00710	0.0743 ± 0.00647	
G40E40	0.0666 ± 0.00508	0.0658 ± 0.00497	0.0739 ± 0.00589	0.0642 ± 0.00479	
G40 E60	0.0692 ± 0.00485	0.0625 ± 0.00456	0.0688 ± 0.00624	0.0647 ± 0.00514	
G60	0.0900 ± 0.00872	0.0944 ± 0.00964	0.1017 ± 0.01172	0.0547 ± 0.00346	
G60E20	0.0629 ± 0.00479	0.0719 ± 0.00629	0.1029 ± 0.01057	0.1009 ± 0.01137	
G60 E40	0.0992 ± 0.00756	0.1027 ± 0.00901	0.1172 ± 0.01264	0.1176 ± 0.01166	
G80	0.1596 ± 0.01495	0.1150 ± 0.01172	0.1207 ± 0.01363	0.0780 ± 0.00774	
G80 E20	0.1022 ± 0.01051	0.1227 ± 0.01490	0.0941 ± 0.00941	0.0874 ± 0.00843	
G99	0.0711 ± 0.00635	0.0669 ± 0.00618	0.3225 ± 0.05814	0.0721 ± 0.00658	
G1.45 E80	0.0687 ± 0.00370	0.0698 ± 0.00491	0.0801 ± 0.00774	0.0706 ± 0.00502	
ANOVA	26.67	3.79	34.64	14.65	
P value	<0.001 HS	0.001 HS	<0.001 HS	<0.001 HS	
Clinical isolates	<i>Enterococcus faecalis</i>	<i>Acinetobacter baumannii</i>	<i>Proteus mirabilis</i>	MRSA	Biofilm producing MRSA
G20	0.0880 ± 0.00946	0.0887 ± 0.00774	0.1021 ± 0.00964	0.0958 ± 0.00831	0.1102 ± 0.00957
G20 E20	0.0857 ± 0.00803	0.0790 ± 0.06501	0.1007 ± 0.01167	0.0877 ± 0.00826	0.1197 ± 0.01160
G20 E40	0.0864 ± 0.00843	0.0729 ± 0.00600	0.0898 ± 0.00906	0.0896 ± 0.00853	0.0818 ± 0.00693
G20 E60	0.0710 ± 0.00502	0.0748 ± 0.00554	0.1101 ± 0.01039	0.0684 ± 0.00479	0.1338 ± 0.01231
G20 E80	0.0677 ± 0.00370	0.0688 ± 0.00491	0.0801 ± 0.00773	0.0706 ± 0.00507	0.0924 ± 0.00850
G40	0.0935 ± 0.00958	0.0910 ± 0.00831	0.0987 ± 0.01045	0.0968 ± 0.00876	0.1115 ± 0.01207
G40 E20	0.0735 ± 0.00745	0.0731 ± 0.00606	0.0750 ± 0.00710	0.0746 ± 0.00647	0.0929 ± 0.00797
G40E40	0.0686 ± 0.00508	0.0659 ± 0.00497	0.0739 ± 0.00587	0.0644 ± 0.00479	0.1105 ± 0.00883
G40 E60	0.0653 ± 0.00485	0.0635 ± 0.00456	0.0689 ± 0.00624	0.0642 ± 0.00514	0.0761 ± 0.00664
G60	0.0940 ± 0.00872	0.0945 ± 0.00964	0.1027 ± 0.01172	0.1039 ± 0.01137	0.1315 ± 0.00910
G60E20	0.0619 ± 0.00479	0.0719 ± 0.00628	0.1039 ± 0.01057	0.0780 ± 0.00776	0.0858 ± 0.00681
G60 E40	0.0992 ± 0.00756	0.1027 ± 0.00900	0.1172 ± 0.01254	0.1076 ± 0.01166	0.1065 ± 0.00947
G80	0.1586 ± 0.01495	0.1151 ± 0.01172	0.1217 ± 0.01363	0.0547 ± 0.00346	0.0837 ± 0.00733
G80 E20	0.1025 ± 0.01051	0.1227 ± 0.01480	0.0941 ± 0.00943	0.0884 ± 0.00843	0.0961 ± 0.00970
G99	0.0721 ± 0.00635	0.0668 ± 0.00618	0.3215 ± 0.05814	0.0731 ± 0.00658	0.0607 ± 0.00741
G1.45 E80	0.0687 ± 0.00370	0.0698 ± 0.00491	0.0801 ± 0.00774	0.0706 ± 0.00502	0.0924 ± 0.00860
ANOVA	26.67	3.79	34.64	14.65	22.28
P value	<0.001 HS	0.001 HS	<0.001 HS	<0.001 HS	<0.001 HS

Optical density for positive control = 0.3985 ± 0.04720.

Optical density for negative control = 0.0346 ± 0.01213.

MRSA, Methicillin-resistant *Staph.aureus*.

Table 2. Assessment of bactericidal activity of different glycerol formulations on reference ATCC strains and clinical isolates.

Variables	<i>Enterococcus faecalis</i>	<i>Acinetobacter baumannii</i>	<i>Proteus mirabilis</i>	<i>Staph. aureus</i>	<i>Enterococcus faecalis</i>	<i>Acinetobacter baumannii</i>	<i>Proteus mirabilis</i>	MRSA	Biofilm producing MRSA
G20	✓	✓	✓	✓	✓	✓	✓	✓	✓
G20 E20	✓				✓				✓
G20 E40	✓				✓				✓
G20 E60		✓				✓			✓
G20 E80									✓
G40	✓				✓				✓
G40 E20	✓			✓	✓			✓	✓
G40E40	✓				✓				✓
G40 E60									✓
G60	✓				✓				✓
G60E20				✓				✓	✓
G60 E40		✓				✓			✓
G80									✓
G80 E20	✓			✓	✓			✓	✓
G99	✓	✓		✓	✓	✓		✓	✓
G1.45 E80									✓

MRSA, Methicillin-resistant *Staph.aureus* ✓: positive growth.

In this work glycerol was proved to have varied combination effect with ethanol according to FICI association formula as shown in Table 2. Drugs combination can decrease the needed dose of each drug, reducing its toxic effects. According to FICI formula combination of glycerol and ethanol showed synergistic effect against *E. faecalis* (reference and clinical isolate) and Biofilm producing MRSA. Additive effect was detected against *P. mirabilis*, *S. aureus* (reference strains and clinical isolates) and nonbiofilm producing MRSA. Regarding *A. baumannii*, both reference and clinical isolates displayed indifference. No antagonism was detected on combination of glycerol and ethanol against either reference or clinical strains.

Extra- and intra-cellular concentration of G3P were positively correlated with used glycerol concentration added to the bacterial cultures with high statistically significant difference (P value < 0.001) (Fig. 1).

The best tolerability was detected in Group C by 100% followed by Group D by 92.3% with no statistically significant difference between both groups (P value 0.07). There was insignificant difference between ratings of skin tolerance among groups B (79.5%) and group D. Group A showed the least tolerability by 33.3% which was significant statistically in comparison to other groups (Table 3).

4. Discussion

Various bacterial infections can be transmitted through contaminated hands. Globally, about 2 million persons acquire nosocomial infections with 90,000 deaths from HCWs contaminated hands [6]. Hand hygiene is the cornerstone of infection prevention and control in hospitals. However, practice

compliance remains the main problem [12]. The minimal concentration of glycerol needed to protect hands remains unclear with reported variable glycerol-alcohol interaction. Here we are trying to evaluate the tolerance of HCWs to different glycerol concentrations. In this study, high concentrations of glycerol were mostly effective in bacterial growth inhibition with better skin tolerability either alone or in combination with ethanol with no antagonism observed in glycerol-alcohol combination against tested strains. Our results are in agreement with Stout and McKessor [13], showing glycerin and glycerin-based products as effective antimicrobial agents with minimal side effects. The topical application of glycerin could inhibit bacterial growth with maintenance of skin integrity. In many previous studies, wounds infected with *Pseudomonas aeruginosa*, *Escherichia coli*, *Str. pyogenes*, *S. aureus*, and MRSA, were treated with glycerin and showed marked reduction in their microbial load [14–16].

In addition, Zich [17], found low glycerol concentration (<4%) could enhance bacterial growth while as concentration was increased growth was inhibited. Also, Wing et al. [18]; Szymanowska-Powalowska [19], reported glycerol as osmotically active substance with a significant effect on the osmotic capacity of fermentation medium limiting growth of microorganisms. On the same line, our research detected G20 E80 and Glycerol 80% as excellent antiseptic substitute to E 80G1.45 with higher tolerability even with allergic conditions matching results of Youssef et al. [20]; Alajlan et al. [9]. On the other hand, Suchomel et al. [21], stated glycerol could form sticky agglomerates reducing its antimicrobial efficacy in some laboratory-based microbiological investigations.

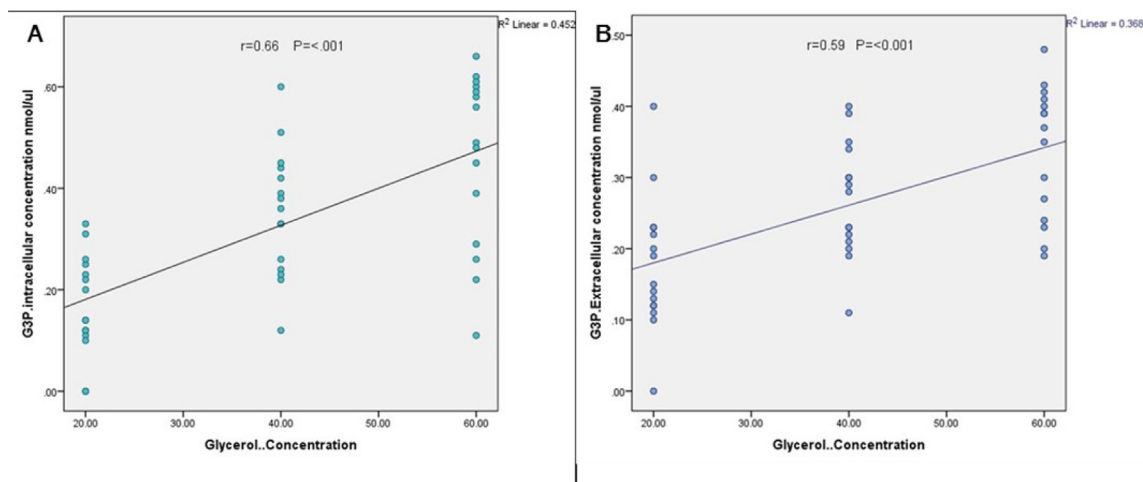


Fig. 1. A&B: Assessment of G3P metabolism among strains; measuring intracellular (A) and extracellular G3P concentrations (B).

Table 3. Tolerability to different glycerol concentrations in ethanol based hand rub formulations among 39 HCWs included in the study.

Variables	Group A (Without glycerol) N (%)	Group B (Glycerol 1.45% Ethanol 80%) N (%)	Group C (Glycerol 20% Ethanol 80%) N (%)	Group D (Glycerol 80%) N (%)
Tolerability:				
Tolerable	13 (33.3)	31 (79.5)	39 (100.0)	36 (92.3)
Not tolerable	26 (66.7)	8 (20.5)	0 (0.0)	3 (7.7)
χ^2	57.64			
P value	<0.001 HS			
Pairwise comparison	P1: <0.001 HS P4: 0.10 NS P2: <0.001 HS P5: 0.002 S P3: <0.001 HS P6: 0.07 NS			

P1: Comparison of group A Versus group B.

P2: Comparison of 0% group A Versus Glycerol group C.

P3: Comparison of group A Versus group D.

P4: Comparison of group B Versus group C.

P5: Comparison of group B Versus group D.

P6: Comparison of group C Versus group D.

χ^2 : Chi square test.

In the present study, no visible growth was detected at formulations G80, G20 E80 and G1.45 E80 (bactericidal effect) followed by G40 E60. This is consistent with the research by Dr. David P. Mackie from Netherlands, who reported that using 85% glycerin solutions had bactericidal effects [22]. We should keep in our minds that light scattering in spectrophotometry could not differentiate between living and dead bacterial cells which could explain the increased optical density measured by spectrophotometer after addition of some glycerol formulations despite no visible growth was detected on subsequent culture.

The bacterial toxic effect of glycerol could be explained as most organisms cannot accommodate dehydrated state produced by glycerol so they either die or become dormant. When glycerol enters bacterial cells, osmotic pressure rises with weakening of cell membranes or even lysis depending on cell wall resistance varying from one species to another depending on cell wall structure which explains increased resistance of Gram positive bacteria to glycerol especially biofilm producing strains [16].

In this study, Gram positive bacteria; *Enterococci* and MRSA especially biofilm producing isolate were resistant to most concentrations except G80, G20 E80 and G1.45 E80. On the other hand, *P. mirabilis* as Gram negative organism was sensitive to all formulations except the lowest G20%. Biofilms are added barriers increasing bacterial resistance to glycerol, this explains why biofilm forming MRSA was inhibited by G99.9 as mentioned by Nedzesky et al. [23]. On the opposite side, previous studies declared that glycerol metabolism promotes biofilm formation by different types of bacteria [24].

In current research, addition of ethanol in optimum concentrations (60%–80%) could be responsible for observed bactericidal effect of mixed

glycerol formulations. An interesting point in our results; 99.9% Glycerol containing formulation had less bactericidal effect compared to 80% and 60% concentrations. The limited permeability of highly viscous 99% glycerol could reflect the importance of dilution in enhancing its bactericidal activity especially with reduced cell permeability. At the same time, undiluted glycerin was to some extent irritating. In previous study done by Becker et al. [25], there were no treatment effects when 99% glycerin was applied.

Treatment of bacterial cultures with glycerol formulations was positively correlated with extra and intracellular concentrations of G3P with subsequent increase in bacterial growth followed by growth inhibition with toxins accumulation matching results obtained by other studies; Liu et al., and Possik et al. [4,26], They recommended the modulation of G3P metabolic pathways for the control of drug resistant microbial infections.

Our study involved many strength points on testing HCWs skin tolerability to different formulations including; real-life evaluation conditions with 7 days duration for each formulated hand rub trial to prevent cumulative skin adverse effects of different formulations [27,28]. Also the antibacterial effect of glycerol was assessed at the incubation of 37 °C which is mimic to body temperature; the best for optimum microbial growth. However, our study was conducted on only nine bacterial strains. More variant bacterial strains with more variable antiseptic formulations should be tested to evaluate our obtained results.

4.1. Conclusions

Skin damage plus inconsistent availability of good and cheap hand rub are important barriers for hand hygiene compliance increasing rate of hospital

acquired infections. Glycerol is a cheap, tolerable and renewable antibacterial agent. However, few studies were concerned with its formulations. We recommend glycerol 80% as ideal antiseptic agent with enhanced tolerability. However appropriate contact time requires concentrated study. G20 E80 formulation recorded better tolerability in comparison to WHO recommended G1.45 E80 formulation with 100% bactericidal non-antagonistic activity.

Glycerol-3-phosphate is important metabolic intermediate with a questionable impact on bacterial growth and virulence. Further *in vivo* and *in vitro* studies are needed. Our findings provide evidence to indicate G3P/Glycerol as a drug target for the treatment of multidrug resistant bacterial infections. Future studies on larger scale should support or reject our results regarding the most effective glycerol concentration with the maximum bactericidal activity tested for different microbes.

Conflicts of interest

No conflict of interest.

Acknowledgements

No Funding.

References

- [1] Ortiz-Padilla M, Portillo-Calderón I, Velázquez-Escudero A, Rodríguez-Baño J, Pascual A, Rodríguez-Martínez JM, et al. Effect of glycerol on fosfomycin activity against *Escherichia coli*. *Antibiotics* 2022;11:1612.
- [2] Alashek F, Keshe M, Alhassan G. Preparation of Glycerol derivatives by entered glycerol in different chemical organic reactions: a review. *Results Chem* 2022;4(20):100359.
- [3] Wound TK. Healing: cost efficient patient care using glycerol-based hydrogel technology. Continuing education paper supported by Southwest Technologies, Inc; 2011.
- [4] Liu Y, Sun W, Ma L, Xu R, Yang C, Xu P, et al. Metabolic mechanism and physiological role of Glycerol 3-Phosphate in *Pseudomonas aeruginosa* PAO1. *mBio* 2022;13:026244. 22.
- [5] Meneguetti MG, Laus AM, Ciol MA, Auxiliadora-Martins M, Basile-Filho A, Gir E, et al. Glycerol content within the WHO ethanol-based handrub formulation: balancing tolerability with antimicrobial efficacy. *Antimicrob Resist Infect Control* 2019;8:1–8.
- [6] World Health Organization. Guide to hygiene and sanitation in aviation. World Health Organization; Geneva: 2009.
- [7] Tille P. Bailey & Scott's diagnostic microbiology-E-Book. Elsevier Health Sciences; Canada: 2015.
- [8] Wayne PA. Clinical and Laboratory Standards Institute: performance standards for antimicrobial susceptibility testing; 20th informational supplement. CLSI document M100-S20. 2010.
- [9] Karthik L, Kumar G, Keswani T, Bhattacharyya A, Chandar SS, Bhaskara Rao KV. Protease inhibitors from marine actinobacteria as a potential source for antimalarial compound. *PLoS One* 2014;9:e90972.
- [10] Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clin Infect Dis* 2009;49(11):1749–55. <https://doi.org/10.1086/647952>.
- [11] Xie J, Ye J, Cai Z, Luo Y, Zhu X, Deng Y, et al. GPD1 enhances the anticancer effects of metformin by synergistically increasing total cellular glycerol-3-phosphate. *Cancer Res* 2020;80:2150–62.
- [12] Ghorbani A, Sadeghi L, Shahrokhi A, Mohammadpour A, Addo M, Khodadadi E. Hand hygiene compliance before and after wearing gloves among intensive care unit nurses in Iran. *Am J Infect Control* 2016;44:e279–81.
- [13] Stout EI, McKessor A. Glycerin-based hydrogel for infection control. *Adv Wound Care* 2012;1:48–51.
- [14] Oliveria-Gandia M, Davis SC, Mertz PM. Can occlusive dressing composition influence proliferation of bacterial wound pathogens? *Wounds* 1998;10:4–11.
- [15] Ectors NL. Tissue banks, university hospital leuven, katholieke universiteiten leuven. Leuven, Belgium: Elsevier; 2007. p. 305–4179.
- [16] Saegeman V, Ectors N, Lismont D, Verduyck B, Verhaegen J. Short and long term bacterial inhibiting effect of high concentrations of glycerol used in the preservation of skin allografts. *Burns* 2008;34:205–11.
- [17] Zich DB. Glycerol for improving solubility and evaluation of antimicrobial activity for natural products. In: A thesis submitted to the faculty of the graduate school at the university of North Carolina at Greensboro in partial fulfillment of the requirements for the degree master of science; 2016.
- [18] Wing KM, Phillips MA, Baker AR, Burke MK. Consequences of cryopreservation in diverse natural isolates of *Saccharomyces cerevisiae*. *Genome Biol Evol* 2020;12:1302–12.
- [19] Szymanowska-Powalowska D. The effect of high concentrations of glycerol on the growth, metabolism and adaptation capacity of *Clostridium butyricum* DSP1. *Electron Biotechnol* 2015;18:128–33.
- [20] Youssef R, Hafez V, Elkholy Y, Mourad A. Glycerol 85% efficacy on atopic skin and its microbiome: a randomized controlled trial with clinical and bacteriological evaluation. *J Dermatol Treat* 2021;32:730–6.
- [21] Suchomel M, Weinlich M, Kundi M. Influence of glycerol and an alternative humectant on the immediate and 3-hours bactericidal efficacies of two isopropanol-based antiseptics in laboratory experiments *in vivo* according to EN 12791. *Antimicrob Resist Infect Control* 2017;6:1–5.
- [22] Mackie DP. The Euro Skin Bank: development and application of glycerol-preserved allografts. *J Burn Care Rehab* 1997; 18:PS7–9.
- [23] Nedzesky N, Matela A, Lavelle T, Carson B. The effects of glycerol on biofilm production. *J Stud Res* 2021;10(4). <https://doi.org/10.47611/jsrhs.v10i4.1834>.
- [24] Tapia NC, den Besten HMW, Abee T. Glycerol metabolism induces *Listeria monocytogenes* biofilm formation at the air-liquid interface. *Int J Food Microbiol* 2018;273:20–7.
- [25] Becker LC, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler DC, et al. Safety assessment of glycerin as used in cosmetics. *Int J Toxicol* 2019;38:6S–22S.
- [26] Possik E, Schmitt C, Al-Mass A, Bai Y, Côté L, Morin J, et al. Phosphoglycolate phosphatase homologs act as glycerol-3-phosphate phosphatase to control stress and healthspan in *C. elegans*. *Nat Commun* 2022;13:177.
- [27] Rundle CW, Presley CL, Militello M, Barber C, Powell DL, Jacob SE, et al. Hand hygiene during COVID-19: recommendations from the American contact dermatitis society. *J Am Acad Dermatol* 2020;83:1730–7.
- [28] Larson E, Girard R, Pessoa-Silva CL, Boyce J, Donaldson L, Pittet D. Skin reactions related to hand hygiene and selection of hand hygiene products. *Am J Infect Control* 2006;34: 627–35.