The Immunomodulatory, Antimicrobial and Bactericidal Efficacy of Commonly Used Commercial Household Disinfectants, Sterilizers and Antiseptics *in Vitro*: Putative Anti-Inflammatory Infection Control Mechanisms and Comparative Biochemical Analysis of the Microbial Growth of Gram-Positive Bacteria

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Abstract Background: Immunomodulatory/anti-inflammatory and microbial infection control means are major benchmarks that characterize the spiral evolution of awareness of public health safety in modern society. This issue is substantiated with burgeoning number of cases of microbial contamination and/or infection in myriad healthcare settings, at the hospital, and even at home. This study investigates the antimicrobial/bactericidal effects of commercially available disinfectants, sterilizers, antiseptics, and chlorhexidine-containing detergents on the growth of saprophytic and pathogenic gram-positive bacteria *in vitro*. It is an unprecedented wide canopy enveloping standardized comparative assessments of the antimicrobial efficiency of consumer-targeted household detergents, curbing and containing microbial infection, inflammation and contamination propensity. **Methods:** Given the medical significance and impact of public infection control, we have meticulously examined at least 22 different detergents categorized into four classes (each category comprises a variety of commercially available products commonly used by the public): i) Class A – *Daily Mouthwash*; ii) Class B – *Toilet Bowl Cleaners/Bleaches/Sanitizers*; iii) Class C – *Surface and Floor Mopping Cleaners/Detergents*; and iv) Class D – *Hand and Body Wash Gels*. Whilst the canonical menu of active ingredients varies among those aforementioned classes, antimicrobial components are well established. **Results:** Regarding Class A, the most effective against *Bacillus subtilis* is ‘Colgate Plax Mouthwash’; *Enterococcus faecalis* are ‘Sensodyne Pronamel Mouthwash’ and ‘Oral-B Pro-Expert Mouthwash’; *Staphylococcus aureus* is ‘Colgate Plax Mouthwash’; *Streptococcus pyogenes* is ‘Colgate Plax Mouthwash’; and *Streptococcus agalactiae* is ‘Sensodyne Pronamel Mouthwash’. Regarding Class B, the most effective against *B. subtilis* is ‘Carrefour Nettoyant Disinfectant’; *E. faecalis* are ‘WC Net Bleach Gel’ and ‘Carrefour Nettoyant Disinfectant’; *S. aureus* are ‘Carrefour Nettoyant Disinfectant’ and ‘Harpic Power Plus Disinfectant’; *S. pyogenes* is ‘WC Net Bleach Gel’; and *S. agalactiae* is ‘WC Net Bleach Gel’. Regarding Class C, the most effective against *B. subtilis* is ‘Vim Cream Multipurpose Fast Rinsing’; *E. faecalis* are ‘Dettol Antiseptic/Disinfectant’ and ‘Spartan Septol Antiseptic/Disinfectant’; *S. aureus* is ‘Ajax Fete des Fleurs’; *S. pyogenes* is ‘Dettol Antiseptic/Disinfectant’; and *S. agalactiae* is ‘Dettol Antiseptic/Disinfectant’. Regarding Class D, the most effective against *B. subtilis*, *E. faecalis*, *S. aureus*, *S. pyogenes*, and *S. agalactiae* is unprecedentedly the ‘HiGee Hand and Body Wash Gel’. **Conclusions:** These results emphatically confirm and verify immunomodulatory infection control variations in the antimicrobial/anti-inflammatory effectiveness of household antiseptics and disinfectants ameliorating the growth of saprophytic and pathogenic gram-positive bacteria in culture.

Keywords: antimicrobial, anti-inflammation, antiseptics, bactericidal, bleaches, disinfectants, disk diffusion, gram-positive bacteria, household detergents, immunomodulation, infection control, novobiocin, sterilizers

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1. Introduction

Annually, and as is the norm with the dawn of modern society and its socio-economical privileges, the commercial market presumptively promotes a humongous amount of infection control oriented advertisements related to household disinfectants, antiseptics, and cleaning detergents [1]. The categorization of commercially available detergents into disinfectants and/or antiseptics has been ergonomically crucial in determining antimicrobial, antifungal, and anti-inflammatory propensities and efficacies across a wide spectrum of pathogenic and saprophytic microorganisms [1,2,3]. Despite the innumerate commercial detergents inundating domestic and international markets, little or perhaps negligible is what is currently known about the microbial canopy likely to be regulated, controlled and affected, and, more importantly, their durability and measurable preemptive efficacy [4,5].

In the past several recent years, there has been a backlog of unprecedented interest in the root causes of many house- and hospital-borne microbial-associated illnesses and disorders [6,7,8]. Consequently, the market has been spirally flooded with antimicrobial household products that have been incessantly introduced to ostensibly try and curb bacterial infections and contaminations, and that is certainly recognized an attempt to evaluate and measure the pervasiveness and effectiveness of infection control in public healthcare settings, points of care, households, and clinics [9,10]. By definition, according to the World Health Organization (WHO), Environmental Protection Agency (EPA), and Centers for Disease Control and Prevention (CDC), ‘antimicrobial’ products are substances, or compounds, or herein mixtures of substances that are “*used to destroy or suppress the growth of harmful microorganisms on household surfaces [inanimate or otherwise]*.”

It is now established that disinfectant and antiseptic antimicrobial products contain, as reported, approximately 300 different active ingredients, and are marketed in a myriad of formulations, including sprays, liquids, gels, concentrated powders, and, sometimes, gases [11,12,13,14,15]. Furthermore, it is estimated that consumers, domestic and international, spend what is approximately estimated at billions of dollars each year on a variety of different disinfectant and antiseptic products. With this astronomical attention given to antimicrobial products, many consumers incessantly wonder as to what products are most likely purchased, and whether those products are not only safe but also actually effective and durable [16,17,18]. The assumption that household products are effectively antimicrobial in nature has yet to be verified and ascertained.

Many studies have attempted to measure the antimicrobial and anti-inflammatory potentials of household detergents [19-25]. To the best of our knowledge, none of the aforementioned investigations and farther afield has offered a canopy of analytical measurements on a wide spectrum of saprophytic and pathogenic microorganisms, whilst covering the major household products of myriad brands available on the market to the extent of assessing many gram-positive and

gram-negative bacteria, including: *Bacillus subtilis*, *Citrobacter koseri*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Escherichia coli*, *E. coli* ESBL, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella sonnei*, *Staphylococcus aureus*, *Streptococcus pyogenes* (Group A *Streptococcus*), and *Streptococcus agalactiae* (Group B *Streptococcus*), in addition to the highly pathogenic fungus, *Candida albicans*.

Considering the necessity of infection control, this study is a pioneer in determining the antimicrobial effect of virtually most of the commercially available disinfectants and antiseptics available in the market, and is meticulously designed to reflect upon not only the accuracy and validity of information inundating consumers, but also the futuristic endeavors in terms of addressing public health concerns and adopting hygienic approaches to containing pathogenic microorganisms of medical importance in various household setups [1,26,27,28,29,30]. Safety of all house members, especially children, remains a concern in modern societies with burgeoning pollution and microbial contaminations. The work therein reported is meant to address those safety issues pertaining to hygiene and welfare, and presents to eager and perhaps unknowing consumers calculated, precise and definitive scientifically-based choices for safe and healthy selections.

2. Materials and Methods

2.1. Analytical Chemicals and Reagents

Unless otherwise indicated, chemicals of the highest analytical purity and grade were purchased from Sigma-Aldrich Corporation, according to standards provided by the American Chemical Society (ACS).

2.2. Preparatory Methods and Design

2.2.1. Bacterial Strains

All bacterial strains studied in this report were gram-positive and included: Gram-positive rods – *Bacillus subtilis* (*B. subtilis*– aerobe); and gram-positive cocci or diplococci – *Enterococcus* Group D (EGD; *E. faecalis*– facultative anaerobe); *Staphylococcus aureus* (*S. aureus* – facultative anaerobe); Group A *Streptococcus* (GAS; *S. pyogenes*– facultative anaerobe); and Group B *Streptococcus* (GBS; *S. agalactiae* – facultative anaerobe). All clinical bacterial specimens that were properly collected and stored were gratis of the Clinical Laboratory Medicine departments at Hammoud Hospital University Medical Center (HHUMC; Saida, Lebanon), and Al-Makassed General Hospital University Medical Center (MGHUMC; Beirut, Lebanon).

2.2.2. Disk Diffusion Method

Prior to experimental use, all bacterial strains were cultured, grown and maintained on nutrient agar medium. The widely-used Muller-Hinton plates were seeded with bacterial inoculums (5×10^8 CFU/ml). Sterile filter paper disks (Whatman n° 1, 5 mm in diameter) were totally dipped in product undiluted or with serial dilutions (2, 4, 8, 16, and 32 fold), using ice-cold, pre-equilibrated

phosphate buffered saline (PBS) buffer. Petri dishes were pre-seeded with 0.5 ml of inoculums and product disks were then placed on the seeded agar plates. All types of commercial products were tested in triplicate. The plates were then kept at 4°C for 1 h for diffusion of product, thereafter incubated at 37°C for 24 h. prior to collecting experimental observations.

2.3. Statistical Analysis and Data Handling

Statistical analysis of the results was completed using Microsoft Office Excel 2013. Experimental results were expressed as mean $\pm$ SEM of at least three independent experiments. Statistical analysis was performed by one-way analysis of variance (ANOVA), followed by *post hoc* Tukey's test to determine significance of mean separation among treatments. Longitudinal optimal differentiation between data sets was also determined and confirmed by

Student's *t*-test. The *a priori* level of significance at 95% confidence was considered valid at $P \leq 0.05$. Further statistical significance is also verified at $P \leq 0.01$ and $P \leq 0.001$, at 99% and 99.9% levels of confidence. Significant variations were indicated with single (*), double (**), or triplet (***) stars for $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

3. Results

All experimental results therein reported are typical observations of at least three (3) different experiments. The various classes used (A, B, C, and D) are grouped according to intended usage as a household modality, and hence variations within any given class are clearly indicated.

Table 1. The inhibition zone diameter methodological analysis of the effect of daily mouthwash (Class A) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	control ddH ₂ O	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class A – Daily Mouthwash[§]									
Sensodyne Pronamel Mouthwash (Disinfectant)									
Active ingredients – Sodium fluoride (0.05%).									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI *	NI	–	–	–	–	–	–	16.00 $\pm$ 0.45
<i>Enterococcus</i> Group D (<i>E. faecalis</i>)	NI	NI	24.33 $\pm$ 0.35	18.00 $\pm$ 0.58	13.33 $\pm$ 0.96	5.00 $\pm$ 2.87	–	–	17.00 $\pm$ 0.58
<i>Staphylococcus aureus</i>	NI	NI	20.67 $\pm$ 0.19	19.00 $\pm$ 0.05	14.33 $\pm$ 0.38	9.33 $\pm$ 0.38	7.33 $\pm$ 0.38	6.67 $\pm$ 0.35	36.00 $\pm$ 1.25
<i>Streptococcus</i> Group A (GAS; <i>S. pyogenes</i>)	NI	NI	15.67 $\pm$ 0.19	13.33 $\pm$ 0.96	8.67 $\pm$ 2.50	4.00 $\pm$ 2.31	3.33 $\pm$ 1.92	–	30.00 $\pm$ 1.57
<i>Streptococcus</i> Group B (GBS; <i>S. agalactiae</i>)	NI	NI	41.33 $\pm$ 5.00	41.00 $\pm$ 5.19	37.33 $\pm$ 7.31	37.33 $\pm$ 7.31	36.00 $\pm$ 8.08	33.33 $\pm$ 9.62	0.00 $\pm$ 0.00

^a Mean value $\pm$ SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.

Table 2. The inhibition zone diameter methodological analysis of the effect of daily mouthwash (Class A) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class A – Daily Mouthwash[§]									
Oral-B Pro-Expert Mouthwash (Disinfectant)									
Active ingredients –Alcohol, Propyl paraben, and Poloxamer 407.									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI *	NI	–	–	–	–	–	–	16.00 $\pm$ 0.45
<i>Enterococcus</i> Group D (<i>E. faecalis</i>)	NI	NI	24.00 $\pm$ 0.58	18.66 $\pm$ 0.38	14.00 $\pm$ 0.33	3.33 $\pm$ 1.92	–	–	17.00 $\pm$ 0.58
<i>Staphylococcus aureus</i>	NI	NI	26.33 $\pm$ 1.35	18.67 $\pm$ 0.19	16.00 $\pm$ 0.33	14.33 $\pm$ 0.19	12.00 $\pm$ 0.33	13.00 $\pm$ 0.88	36.00 $\pm$ 1.25
<i>Streptococcus</i> Group A (GAS; <i>S. pyogenes</i>)	NI	NI	11.67 $\pm$ 0.51	11.33 $\pm$ 1.07	11.00 $\pm$ 1.15	–	–	–	30.00 $\pm$ 1.57
<i>Streptococcus</i> Group B (GBS; <i>S. agalactiae</i>)	NI	NI	22.67 $\pm$ 1.01	18.67 $\pm$ 0.51	17.33 $\pm$ 1.02	11.67 $\pm$ 1.26	–	–	0.00 $\pm$ 0.00

^a Mean value $\pm$ SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.

Table 3. The inhibition zone diameter methodological analysis of the effect of daily mouthwash (Class A) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class A – Daily Mouthwash [§]									
Colgate Plax Mouthwash (Disinfectant)									
Active ingredients – Cetylpyridinium chloride (0.05%, w/w), and Ethanol (7.3%).									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI *	NI	12.00 ± 0.38	10.00 ± 0.33	8.00 ± 1.54	–	–	–	16.00 ± 0.45
<i>Enterococcus</i> Group D (<i>E. faecalis</i>)	NI	NI	10.00 ± 1.20	11.00 ± 2.14	10.00 ± 2.04	10.00 ± 1.92	10.00 ± 1.92	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	50.00 ± 2.57	50.00 ± 2.38	50.00 ± 2.21	50.00 ± 2.52	50.00 ± 2.42	50.00 ± 2.18	36.00 ± 1.25
<i>Streptococcus</i> Group A (GAS; <i>S. pyogenes</i>)	NI	NI	18.33 ± 0.19	17.00 ± 0.15	7.33 ± 2.21	3.00 ± 1.73	–	–	30.00 ± 1.57
<i>Streptococcus</i> Group B (GBS; <i>S. agalactiae</i>)	NI	NI	25.67 ± 0.38	21.00 ± 0.33	14.00 ± 1.20	13.67 ± 0.69	11.00 ± 0.88	10.00 ± 0.67	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

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Table 4. The inhibition zone diameter methodological analysis of the effect of daily mouthwash (Class A) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	control ddH ₂ O	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class A – Daily Mouthwash [§]									
Fresh Burst Listerine Mouthwash (Disinfectant)									
Active ingredients – Thymol (0.064%), Eucalyptol (0.092%), Methyl salicylate (0.092%), and Menthol (0.042%).									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI *	NI	–	–	–	–	–	–	16.00 ± 0.45
<i>Enterococcus</i> Group D (<i>E. faecalis</i>)	NI	NI	–	–	–	–	–	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	14.00 ± 0.38	12.50 ± 0.19	10.00 ± 0.38	7.50 ± 1.45	7.50 ± 1.45	7.00 ± 1.35	36.00 ± 1.25
<i>Streptococcus</i> Group A (GAS; <i>S. pyogenes</i>)	NI	NI	–	–	–	–	–	–	30.00 ± 1.57
<i>Streptococcus</i> Group B (GBS; <i>S. agalactiae</i>)	NI	NI	7.56 ± 0.38	7.50 ± 1.45	5.67 ± 1.71	8.00 ± 1.54	7.67 ± 0.19	7.50 ± 1.45	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.

Table 5. The inhibition zone diameter methodological analysis of the effect of daily mouthwash (Class A) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class A – Daily Mouthwash [§]									
Perio.Kin Chlorhexidina 0.20% (Disinfectant)									
Active ingredients – Chlorhexidine (0.20%).									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI *	NI	7.00 ± 1.56	–	–	–	–	–	16.00 ± 0.45
<i>Enterococcus</i> Group D (<i>E. faecalis</i>)	NI	NI	6.00 ± 1.76	2.33 ± 1.37	–	–	–	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	–	–	–	–	–	–	36.00 ± 1.25
<i>Streptococcus</i> Group A (GAS; <i>S. pyogenes</i>)	NI	NI	20.67 ± 1.71	19.00 ± 0.58	11.00 ± 0.33	7.00 ± 2.03	6.33 ± 1.89	3.67 ± 2.12	30.00 ± 1.57
<i>Streptococcus</i> Group B (GBS; <i>S. agalactiae</i>)	NI	NI	11.00 ± 0.33	9.67 ± 0.19	–	–	–	–	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.

3.1. The Zones of Inhibition of Gram-Positive Bacterium *Bacillus Subtilis*

3.1.1. The Zones of Inhibition of Class A

The effect of daily mouthwash (category Class A) on the microbial growth of *Bacillus subtilis* is given in Tables 1-5. The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.1.2. The Zones of Inhibition of Class B

The effect of toilet bowl cleaners/bleaches/sanitizers (category Class B) on the microbial growth of *Bacillus subtilis* is given in Tables 6-14. The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.1.3. The Zones of Inhibition of Class C

The effect of surface and floor mopping cleaners/detergents (category Class C) on the microbial growth of *Bacillus subtilis* is given in Tables 15-19. The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.1.4. The Zones of Inhibition of Class D

The effect of hand and body wash gels (category Class D) on the microbial growth of *Bacillus subtilis* is given in Tables 20-22. The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.2. The Zones of Inhibition of Gram-Positive Bacterium *Enterococcus Faecalis* (*Streptococcus* Group D)

3.2.1. The Zones of Inhibition of Class A

The effect of daily mouthwash (category Class A) on the microbial growth of *Enterococcus faecalis* given in Tables 1-5. The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.2.2. The Zones of Inhibition of Class B

The effect of toilet bowl cleaners/bleaches/sanitizers (category Class B) on the microbial growth of *Enterococcus faecalis* given in Tables 6-14. The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.2.3. The Zones of Inhibition of Class C

The effect of surface and floor mopping cleaners/detergents (category Class C) on the microbial growth of *Enterococcus faecalis* given in Tables 15-19.

The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.2.4. The Zones of Inhibition of Class D

The effect of hand and body wash gels (category Class D) on the microbial growth of *Enterococcus faecalis* is given in Tables 20-22. The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.3. The Zones of Inhibition of Gram-Positive Bacterium *Staphylococcus Aureus*

3.3.1. The Zones of Inhibition of Class A

The effect of daily mouthwash (category Class A) on the microbial growth of *Staphylococcus aureus* is given in Tables 1-5. The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.3.2. The Zones of Inhibition of Class B

The effect of toilet bowl cleaners/bleaches/sanitizers (category Class B) on the microbial growth of *Staphylococcus aureus* is given in Tables 6-14. The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.3.3. The Zones of Inhibition of Class C

The effect of surface and floor mopping cleaners/detergents (category Class C) on the microbial growth of *Staphylococcus aureus* is given in Tables 15-19. The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.3.4. The Zones of Inhibition of Class D

The effect of hand and body wash gels (category Class D) on the microbial growth of *Staphylococcus aureus* is given in Tables 20-22. The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.4. The Zones of Inhibition of Gram-Positive Bacterium *Streptococcus Pyogenes* (*Streptococcus* Group A)

3.4.1. The Zones of Inhibition of Class A

The effect of daily mouthwash (category Class A) on the microbial growth of *Streptococcus pyogenes* is given in Tables 1-5. The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

Table 6. The inhibition zone diameter methodological analysis of the effect of toilet bowl cleaners/bleaches/sanitizers (Class B) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class B – Toilet Bowl Cleaners/Bleaches/Sanitizers [§]									
WC Net Bleach Gel									
Active ingredients – Aqua, Sodium hypochlorite, Alkyl dimethylamine oxide, Sodium hydroxide, and Sodium laurate.									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI *	NI	11.67 ± 0.83	–	–	–	–	–	16.00 ± 0.45
<i>Enterococcus</i> Group D (<i>E. faecalis</i>)	NI	NI	24.33 ± 1.64	17.33 ± 1.35	8.67 ± 0.19	–	–	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	30.33 ± 0.19	27.00 ± 0.58	17.00 ± 2.33	14.67 ± 0.19	12.67 ± 0.38	9.67 ± 0.19	36.00 ± 1.25
<i>Streptococcus</i> Group A (GAS; <i>S. pyogenes</i>)	NI	NI	33.00 ± 0.58	20.00 ± 1.67	14.67 ± 0.84	11.33 ± 1.07	2.67 ± 1.53	–	30.00 ± 1.57
<i>Streptococcus</i> Group B (GBS; <i>S. agalactiae</i>)	NI	NI	TI 50.00 ± 2.17	TI 50.00 ± 2.28	TI 50.00 ± 1.83	TI 50.00 ± 2.50	TI 50.00 ± 2.42	TI 50.00 ± 2.18	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.

Table 7. The inhibition zone diameter methodological analysis of the effect of toilet bowl cleaners/bleaches/sanitizers (Class B) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class B – Toilet Bowl Cleaners/Bleaches/Sanitizers [§]									
Mr. Muscle Toilet Cleaner Duck									
Active ingredients – Aqua, Tetrasodium EDTA, Butoxydiglycol, C9-11 parath-6, Benzalkonium chloride, Sodium hydroxide, Parfum, Trisodium NTA, 3-(Trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride, Benzyl salicylate, Alcohol, (3-Chloropropyl) trimethoxysilane, Methyl alcohol, Linalool, Lactic acid (2.02 g/100 g), Limonene, and Dimethyl stearamine.									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI *	NI	–	–	–	–	–	–	16.00 ± 0.45
<i>Enterococcus</i> Group D (<i>E. faecalis</i>)	NI	NI	8.00 ± 0.33	8.33 ± 0.45	9.00 ± 1.73	–	–	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	25.33 ± 0.20	23.33 ± 0.38	17.00 ± 1.15	14.33 ± 1.07	12.00 ± 0.67	10.00 ± 0.67	36.00 ± 1.25
<i>Streptococcus</i> Group A (GAS; <i>S. pyogenes</i>)	NI	NI	9.33 ± 0.39	10.00 ± 1.92	–	–	–	–	30.00 ± 1.57
<i>Streptococcus</i> Group B (GBS; <i>S. agalactiae</i>)	NI	NI	8.33 ± 0.19	7.50 ± 1.45	–	–	–	–	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.

Table 8. The inhibition zone diameter methodological analysis of the effect of toilet bowl cleaners/bleaches/sanitizers (Class B) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class B – Toilet Bowl Cleaners/Bleaches/Sanitizers [§]									
Germicidal Bowl Cleanse Spartan Flash									
Active ingredients – Quaternary ammonium chloride, Hydrogen chloride, Non-ionic surfactant, and Corrosion inhibitor.									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI *	NI	10.67 ± 0.19	8.68 ± 0.20	7.32 ± 0.18	–	–	–	16.00 ± 0.45
<i>Enterococcus</i> Group D (<i>E. faecalis</i>)	NI	NI	13.32 ± 0.51	11.33 ± 0.38	–	–	–	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	15.33 ± 0.20	14.00 ± 0.33	10.33 ± 0.69	9.00 ± 0.67	8.50 ± 1.64	8.00 ± 1.53	36.00 ± 1.25
<i>Streptococcus</i> Group A (GAS; <i>S. pyogenes</i>)	NI	NI	20.33 ± 1.34	17.00 ± 2.11	17.00 ± 3.27	14.00 ± 2.69	–	–	30.00 ± 1.57
<i>Streptococcus</i> Group B (GBS; <i>S. agalactiae</i>)	NI	NI	19.33 ± 2.04	14.33 ± 0.77	8.00 ± 0.58	4.33 ± 1.26	–	–	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.

Table 9. The inhibition zone diameter methodological analysis of the effect of toilet bowl cleaners/bleaches/sanitizers (Class B) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class B – Toilet Bowl Cleaners/Bleaches/Sanitizers [§] Carrefour Nettoyant Disinfectant Active ingredients –Hypochlorite, Non-ionic surfactant (< 5%), and Chlorine benzalkonium.									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI *	NI	20.67 ± 0.69	17.67 ± 0.38	15.00 ± 0.67	13.00 ± 1.20	8.33 ± 0.18	–	16.00 ± 0.45
<i>Enterococcus</i> Group D (<i>E. faecalis</i>)	NI	NI	24.33 ± 2.21	22.33 ± 0.19	18.00 ± 0.88	13.67 ± 0.69	10.00 ± 0.88	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	50.00 ± 1.25	50.00 ± 2.22	36.33 ± 3.95	26.67 ± 0.21	22.33 ± 0.50	21.33 ± 0.38	36.00 ± 1.25
<i>Streptococcus</i> Group A (GAS; <i>S. pyogenes</i>)	NI	NI	28.67 ± 1.71	26.33 ± 1.17	22.00 ± 0.57	17.32 ± 0.19	15.68 ± 0.77	11.00 ± 2.14	30.00 ± 1.57
<i>Streptococcus</i> Group B (GBS; <i>S. agalactiae</i>)	NI	NI	45.00 ± 2.88	44.00 ± 3.46	44.00 ± 3.45	23.00 ± 1.04	17.00 ± 1.02	21.00 ± 1.15	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.

Table 10. The inhibition zone diameter methodological analysis of the effect of toilet bowl cleaners/bleaches/sanitizers (Class B) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class B – Toilet Bowl Cleaners/Bleaches/Sanitizers [§] La Croix Sans Javel (Antibacterial) Active ingredients –Anionic surfactants (< 5%), Non-ionic surfactants (< 5%), Hypochlorite, Lactic acid (1.5%), Linalool, Citronellol, Coumarin, and Butylphenyl methylpropional.									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI *	NI	–	–	–	–	–	–	16.00 ± 0.45
<i>Enterococcus</i> Group D (<i>E. faecalis</i>)	NI	NI	8.00 ± 1.53	8.00 ± 1.53	8.00 ± 1.53	8.00 ± 1.53	–	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	31.32 ± 0.69	29.67 ± 0.51	21.67 ± 0.53	17.65 ± 0.20	15.31 ± 0.71	13.30 ± 0.38	36.00 ± 1.25
<i>Streptococcus</i> Group A (GAS; <i>S. pyogenes</i>)	NI	NI	19.33 ± 0.70	18.00 ± 0.67	15.33 ± 0.52	–	–	–	30.00 ± 1.57
<i>Streptococcus</i> Group B (GBS; <i>S. agalactiae</i>)	NI	NI	–	–	–	–	–	–	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.

Table 11. The inhibition zone diameter methodological analysis of the effect of toilet bowl cleaners/bleaches/sanitizers (Class B) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class B – Toilet Bowl Cleaners/Bleaches/Sanitizers [§] Clorox Bleach Rain Clean Active ingredients –Water, Sodium hypochlorite, Sodium cocoate, C.I. pigment green 7 (74260), Fragrance, Lauramine oxide, Myristamine oxide, N-(3-Chloroallyl) hexaminium chloride, Potassium iodide, and Sodium hydroxide.									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI*	NI	9.00 ± 0.12	8.00 ± 1.53	9.00 ± 1.73	–	–	–	16.00 ± 0.45
<i>Enterococcus</i> Group D (<i>E. faecalis</i>)	NI	NI	20.00 ± 3.84	10.00 ± 1.92	10.00 ± 1.92	–	–	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	26.67 ± 0.19	25.67 ± 0.19	23.00 ± 0.34	17.00 ± 0.88	14.31 ± 0.77	12.33 ± 0.84	36.00 ± 1.25
<i>Streptococcus</i> Group A (GAS; <i>S. pyogenes</i>)	NI	NI	21.68 ± 0.84	17.00 ± 0.88	13.67 ± 0.84	12.67 ± 2.11	14.00 ± 2.69	10.33 ± 0.77	30.00 ± 1.57
<i>Streptococcus</i> Group B (GBS; <i>S. agalactiae</i>)	NI	NI	11.33 ± 0.33	10.33 ± 0.19	8.33 ± 0.33	8.00 ± 0.19	10.00 ± 1.92	7.00 ± 1.34	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.

Table 12. The inhibition zone diameter methodological analysis of the effect of toilet bowl cleaners/bleaches/sanitizers (Class B) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class B – Toilet Bowl Cleaners/Bleaches/Sanitizers[§] Harpic Power Plus Disinfectant Active ingredients –Non-ionic surfactants (< 5%), Cationic surfactants (< 5%), Disinfectant, Perfume, and Hydrochloric acid (9 g/100 g).									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI*	NI	9.33 ± 0.51	7.67 ± 0.20	4.67 ± 1.35	2.33 ± 1.35	–	–	16.00 ± 0.45
<i>Enterococcus</i> Group D (<i>E. faecalis</i>)	NI	NI	15.33 ± 1.02	11.67 ± 0.50	2.67 ± 1.53	–	–	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	50.00 ± 1.05	50.00 ± 1.22	43.33 ± 3.85	40.67 ± 5.38	20.33 ± 1.07	18.00 ± 0.33	36.00 ± 1.25
<i>Streptococcus</i> Group A (GAS; <i>S. pyogenes</i>)	NI	NI	23.33 ± 1.92	16.65 ± 0.19	12.33 ± 0.70	12.67 ± 0.83	10.33 ± 0.51	10.67 ± 0.38	30.00 ± 1.57
<i>Streptococcus</i> Group B (GBS; <i>S. agalactiae</i>)	NI	NI	12.33 ± 0.38	8.00 ± 1.53	8.00 ± 1.51	8.00 ± 1.52	8.00 ± 1.45	9.00 ± 1.73	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.**Table 13. The inhibition zone diameter methodological analysis of the effect of toilet bowl cleaners/bleaches/sanitizers (Class B) on the growth of gram-positive bacteria**

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class B – Toilet Bowl Cleaners/Bleaches/Sanitizers[§] Smac Detergent Disinfectant Active ingredients –Anionic surfactant, Amphoteric surfactant, Antioxidant surfactant, Opacifier, Lanolin, Preservative, Anti-bacteria reagent, Hydrogen chloride, Fragrance, and Color.									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI*	NI	9.33 ± 0.76	8.00 ± 1.54	–	–	–	–	16.00 ± 0.45
<i>Enterococcus</i> Group D (<i>E. faecalis</i>)	NI	NI	14.33 ± 0.19	11.00 ± 0.33	9.33 ± 0.50	7.50 ± 1.45	6.00 ± 1.15	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	28.00 ± 1.45	25.68 ± 2.71	13.33 ± 1.01	13.00 ± 0.34	11.33 ± 0.96	9.00 ± 0.58	36.00 ± 1.25
<i>Streptococcus</i> Group A (GAS; <i>S. pyogenes</i>)	NI	NI	26.33 ± 2.50	21.32 ± 1.71	16.00 ± 2.03	11.67 ± 0.20	10.67 ± 0.52	8.00 ± 2.32	30.00 ± 1.57
<i>Streptococcus</i> Group B (GBS; <i>S. agalactiae</i>)	NI	NI	17.00 ± 0.25	12.33 ± 1.57	9.00 ± 1.73	8.00 ± 1.54	7.00 ± 1.35	–	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.**Table 14. The inhibition zone diameter methodological analysis of the effect of toilet bowl cleaners/bleaches/sanitizers (Class B) on the growth of gram-positive bacteria**

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class B – Toilet Bowl Cleaners/Bleaches/Sanitizers[§] La Croix Sans Javel (Antibacterial) Active ingredients –Aqua, Undecan-1-ol, ethoxylated, Methoxy-isopropanol, Butoxy-propanol, Ethanolamine, Benzalkonium chloride, Sodium etidronate, Perfume, Tetrasodium EDTA, Benzisothiazolinone, Dimethicone, Sodium sulfate, Sodium hydroxide, and Colorants.									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI*	NI	–	–	–	–	–	–	16.00 ± 0.45
<i>Enterococcus</i> Group D (<i>E. faecalis</i>)	NI	NI	7.50 ± 1.45	6.00 ± 1.15	–	–	–	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	34.32 ± 1.64	25.67 ± 1.01	23.67 ± 1.71	17.00 ± 0.88	19.67 ± 3.10	13.67 ± 1.20	36.00 ± 1.25
<i>Streptococcus</i> Group A (GAS; <i>S. pyogenes</i>)	NI	NI	24.67 ± 1.68	19.00 ± 1.15	16.67 ± 1.17	10.67 ± 3.36	3.34 ± 1.92	6.00 ± 1.73	30.00 ± 1.57
<i>Streptococcus</i> Group B (GBS; <i>S. agalactiae</i>)	NI	NI	14.00 ± 0.58	10.33 ± 0.84	9.00 ± 1.76	11.00 ± 2.14	13.33 ± 0.50	10.67 ± 0.69	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.

3.4.2. The Zones of Inhibition of Class B

The effect of toilet bowl cleaners/bleaches/sanitizers (category Class B) on the microbial growth of

Streptococcus pyogenes given in Tables 6-14. The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as

a positive control, while absolute methanol is recognized as negative control.

Table 15. The inhibition zone diameter methodological analysis of the effect of surface and floor mopping cleaners/detergents (Class C) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class C – Surface and Floor Mopping Cleaners/Detergents[§]									
Dettol (Antiseptic, Disinfectant)									
Active ingredients – Chloroxylenol (4.8%), Isopropyl alcohol, Pine oil, Caramel, and Castor oil soap.									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI *	NI	8.00 ± 0.15	8.00 ± 1.53	–	–	–	–	16.00 ± 0.45
<i>Enterococcus Group D (E. faecalis)</i>	NI	NI	12.67 ± 0.19	12.00 ± 1.00	12.00 ± 0.25	10.32 ± 0.50	5.31 ± 1.54	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	24.33 ± 0.96	19.33 ± 0.69	17.67 ± 0.84	15.00 ± 0.67	12.00 ± 0.66	12.00 ± 0.32	36.00 ± 1.25
<i>Streptococcus Group A (GAS; S. pyogenes)</i>	NI	NI	35.00 ± 0.25	24.00 ± 0.58	26.67 ± 2.55	19.66 ± 3.60	13.67 ± 0.51	4.00 ± 2.30	30.00 ± 1.57
<i>Streptococcus Group B (GBS; S. agalactiae)</i>	NI	NI	15.33 ± 0.38	13.35 ± 0.38	12.00 ± 0.15	9.00 ± 0.57	–	–	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is >50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.

Table 16. The inhibition zone diameter methodological analysis of the effect of surface and floor mopping cleaners/detergents (Class C) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class C – Surface and Floor Mopping Cleaners/Detergents[§]									
Spartan Septol (Antiseptic, Disinfectant)									
Active ingredients –Parachloro metaxylenol (< 4.8%), Chlorophenol (4.8%), Pine oil, Vegetable soap, Solvent, and Color.									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI *	NI	–	–	–	–	–	–	16.00 ± 0.45
<i>Enterococcus Group D (E. faecalis)</i>	NI	NI	14.00 ± 0.33	13.67 ± 0.58	9.00 ± 0.33	24.00 ± 2.19	6.67 ± 1.92	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	30.33 ± 0.19	30.00 ± 0.12	22.33 ± 0.83	14.67 ± 1.02	11.32 ± 0.18	9.67 ± 0.19	36.00 ± 1.25
<i>Streptococcus Group A (GAS; S. pyogenes)</i>	NI	NI	24.00 ± 2.19	19.00 ± 1.45	16.33 ± 0.18	15.33 ± 0.51	11.33 ± 0.38	12.00 ± 2.31	30.00 ± 1.57
<i>Streptococcus Group B (GBS; S. agalactiae)</i>	NI	NI	9.00 ± 0.33	10.00 ± 0.15	–	–	–	–	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.

Table 17. The inhibition zone diameter methodological analysis of the effect of surface and floor mopping cleaners/detergents (Class C) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class C – Surface and Floor Mopping Cleaners/Detergents[§]									
Vim Cream Multipurpose Fast Rinsing									
Active ingredients – Aqua, Phosphates (< 5%), Anionic surfactants (< 5%), Calcium carbonate, Sodium dodecylbenzene sulfonate, C12-C13 parath-6, Butoxydiglycol, C11-C13 isoparaffin, Sodium cocoate, Parfum, Carbomer, Benzisothiazolinone, and CI 47005.									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI *	NI	17.00 ± 0.05	13.67 ± 0.51	10.33 ± 0.38	8.67 ± 0.19	8.05 ± 0.75	7.00 ± 1.35	16.00 ± 0.45
<i>Enterococcus Group D (E. faecalis)</i>	NI	NI	–	–	–	–	–	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	11.33 ± 0.70	9.67 ± 0.83	9.65 ± 0.70	10.00 ± 0.67	9.00 ± 0.57	–	36.00 ± 1.25
<i>Streptococcus Group A (GAS; S. pyogenes)</i>	NI	NI	–	–	–	–	–	–	30.00 ± 1.57
<i>Streptococcus Group B (GBS; S. agalactiae)</i>	NI	NI	7.50 ± 1.45	7.50 ± 1.40	7.50 ± 1.38	7.50 ± 1.41	7.50 ± 1.45	7.00 ± 1.34	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.

Table 18. The inhibition zone diameter methodological analysis of the effect of surface and floor mopping cleaners/detergents (Class C) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class C – Surface and Floor Mopping Cleaners/Detergents[§] Astonish Vac Maxx (Disinfectant) Active ingredients – Sodium silicate (5 – 15%), Non-ionic surfactants NTA (< 5%), β-Alanine, N-(2-Carboxyethyl)-, N-coco alkyl derivatives, Disodium salts, and 2-Benzyl-4-chlorophenol (Chlorophene ([0.045g/l])).									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI *	NI	–	–	–	–	–	–	16.00 ± 0.45
<i>Enterococcus Group D (E. faecalis)</i>	NI	NI	8.00 ± 1.54	6.00 ± 1.15	–	–	–	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	27.33 ± 4.72	28.00 ± 1.92	17.00 ± 1.01	14.00 ± 1.54	9.67 ± 0.19	8.67 ± 0.38	36.00 ± 1.25
<i>Streptococcus Group A (GAS; S. pyogenes)</i>	NI	NI	21.00 ± 0.33	16.00 ± 0.67	13.67 ± 1.17	8.33 ± 2.54	9.00 ± 1.73	8.67 ± 0.38	30.00 ± 1.57
<i>Streptococcus Group B (GBS; S. agalactiae)</i>	NI	NI	7.00 ± 1.34	7.00 ± 1.45	7.00 ± 1.25	9.00 ± 1.73	9.00 ± 1.73	9.00 ± 1.76	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.

Table 19. The inhibition zone diameter methodological analysis of the effect of surface and floor mopping cleaners/detergents (Class C) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class C – Surface and Floor Mopping Cleaners/Detergents[§] Ajax Fete des Fleurs Active ingredients – Anionic surfactants (< 5%), Non-ionic surfactants, Perfume, Butylphenyl methylpropional, Hexyl cinnamal, Citronellol, Linalool, Geraniol, Glutaral, Methylchloroisothiazolinone, Methylisothiazolinone, and Octylisothiazolinone.									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI *	NI	–	–	–	–	–	–	16.00 ± 0.45
<i>Enterococcus Group D (E. faecalis)</i>	NI	NI	–	–	–	–	–	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	43.32 ± 5.77	37.32 ± 3.67	24.00 ± 2.18	20.00 ± 1.02	17.33 ± 0.83	19.00 ± 19.22	36.00 ± 1.25
<i>Streptococcus Group A (GAS; S. pyogenes)</i>	NI	NI	20.00 ± 3.84	20.00 ± 3.85	17.00 ± 3.27	–	–	–	30.00 ± 1.57
<i>Streptococcus Group B (GBS; S. agalactiae)</i>	NI	NI	8.00 ± 1.14	8.00 ± 1.53	8.00 ± 1.34	8.00 ± 1.57	8.33 ± 0.19	9.33 ± 0.19	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.

3.4.3. The Zones of Inhibition of Class C

The effect of surface and floor mopping cleaners/detergents (category Class C) on the microbial growth of *Streptococcus pyogenes* given in Tables 15-19. The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.4.4. The Zones of Inhibition of Class D

The effect of hand and body wash gels (category Class D) on the microbial growth of *Streptococcus pyogenes* given in Tables 20-22. The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.5. The Zones of Inhibition of Gram-Positive Bacterium *Streptococcus Agalactiae* (*Streptococcus Group B*)

3.5.1. The Zones of Inhibition of Class A

The effect of daily mouthwash (category Class A) on the microbial growth of *Streptococcus agalactiae* is given in Tables 1-5. The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.5.2. The Zones of Inhibition of Class B

The effect of toilet bowl cleaners/bleaches/sanitizers (category Class B) on the microbial growth of *Streptococcus agalactiae* is given in Tables 6-14. The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.5.3. The Zones of Inhibition of Class C

The effect of surface and floor mopping cleaners/detergents (category Class C) on the microbial growth of *Streptococcus agalactiae* is given in Tables 15-

19. The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

Table 20. The inhibition zone diameter methodological analysis of the effect of hand and body wash gels (Class D) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class D – Hand and Body Wash Gels [§] Lifebuoy Hand and Body Wash Gel (Antiseptic) Active ingredients –Glycerine, and Vitamin beads.									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI *	NI	–	–	–	–	–	–	16.00 ± 0.45
<i>Enterococcus Group D (E. faecalis)</i>	NI	NI	–	–	–	–	–	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	–	–	–	–	–	–	36.00 ± 1.25
<i>Streptococcus Group A (GAS; S. pyogenes)</i>	NI	NI	–	–	–	–	–	–	30.00 ± 1.57
<i>Streptococcus Group B (GBS; S. agalactiae)</i>	NI	NI	–	–	–	–	–	–	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.

Table 21. The inhibition zone diameter methodological analysis of the effect of hand and body wash gels (Class D) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class D – Hand and Body Wash Gels [§] HiGeen Hand and Body Wash Gel (Antiseptic) Active ingredients –Glycerine, and Vitamin beads.									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI *	NI	24.67 ± 0.51	17.00 ± 0.88	20.67 ± 1.71	17.66 ± 0.70	12.33 ± 0.84	9.67 ± 0.19	16.00 ± 0.45
<i>Enterococcus Group D (E. faecalis)</i>	NI	NI	11.65 ± 0.50	9.33 ± 0.18	–	–	–	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	50.00 ± 0.50	50.00 ± 0.50	36.34 ± 7.89	17.66 ± 0.50	26.00 ± 6.94	50.00 ± 0.51	36.00 ± 1.25
<i>Streptococcus Group A (GAS; S. pyogenes)</i>	NI	NI	33.00 ± 1.73	22.33 ± 1.50	17.00 ± 1.02	20.00 ± 1.02	9.00 ± 2.65	15.67 ± 0.19	30.00 ± 1.57
<i>Streptococcus Group B (GBS; S. agalactiae)</i>	NI	NI	42.33 ± 4.42	41.67 ± 4.81	29.00 ± 6.08	14.67 ± 1.54	12.67 ± 1.53	4.00 ± 2.31	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.

Table 22. The inhibition zone diameter methodological analysis of the effect of hand and body wash gels (Class D) on the growth of gram-positive bacteria

Microorganism	Inhibition Zone Diameter (mm) ^a								
	Control ddH ₂ O	Control Pure Methanol	Undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Novobiocin (30 µg)
Class D – Hand and Body Wash Gels [§] HiGeen Hand Sanitizer Gel (Antibacterial) Active ingredients –Myristic acid, Lauric acid, Potassium hydroxide, Glycerine, and Thymol.									
Gram-Positive Bacteria									
<i>Bacillus subtilis</i>	NI *	NI	–	–	–	–	–	–	16.00 ± 0.45
<i>Enterococcus Group D (E. faecalis)</i>	NI	NI	–	–	–	–	–	–	17.00 ± 0.58
<i>Staphylococcus aureus</i>	NI	NI	10.00 ± 0.33	9.00 ± 0.51	8.00 ± 0.50	8.00 ± 1.54	8.00 ± 1.51	8.33 ± 0.50	36.00 ± 1.25
<i>Streptococcus Group A (GAS; S. pyogenes)</i>	NI	NI	–	–	–	–	–	–	30.00 ± 1.57
<i>Streptococcus Group B (GBS; S. agalactiae)</i>	NI	NI	10.32 ± 0.51	8.33 ± 0.19	7.00 ± 1.35	7.00 ± 1.42	7.00 ± 1.32	8.00 ± 1.53	0.00 ± 0.00

^a Mean value ± SEM, *n* = 3 (the zone of inhibition [mm] including disk of 5 mm in diameter).

* NI = No Inhibition; TI = Total Inhibition (the zone of inhibition [mm] including disk of 5 mm in diameter is > 50 mm); DF = Dilution Factor.

[§] Commercial brands are disclosed in accordance with ethical and propriety issues.

3.5.4. The Zones of Inhibition of Class D

The effect of hand and body wash gels (category Class D) on the microbial growth of *Streptococcus agalactiae* is given in Tables 20-22. The inhibitory effect of the commonly used antibiotic novobiocin (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.6. The Comparative Analytical Assessment of Various Household Disinfectants

Descending order of resistance to germicidal chemicals is an integral component of identifying the antimicrobial efficacy of household detergents and disinfectants. This hierarchy considers broad classifications of microbial categories and is considered a rough guide to general susceptibility patterns of microorganisms to disinfectants, as shown in Figure 1.

Figure 1. Descending order of resistance to germicidal chemicals. This hierarchy considers broad classifications of microbial categories. It is considered a rough guide to general susceptibility patterns of microorganisms to disinfectants. (Adapted, courtesy of: Favero, M.S.; Bond, W.V. Chemical disinfection of medical and surgical materials. In: Block, S.S.; ed. Disinfection, sterilization and preservation. 4thed. Philadelphia: Lea and Febiger, 1991, 621.)

Figure 2. Depictive comparative assessment of the antimicrobial efficacy of various detergents against gram-positive *Bacillus subtilis* bacteria, as compared with novobiocin (30 µg). The zone of inhibition of novobiocin was set as a reference (lane 24; horizontal straight line), and that for absolute methanol (MetOH) is shown in lane 23, and all values of the zones of inhibition at undiluted concentrations of disinfectant/sterilizer/antiseptic were compared against those references (Lanes 23 and 24). Lanes 1 – 5 represent Class A (Daily Mouthwash); Lanes 6 – 14 represent Class B (Toilet Bowl Cleaners/Bleaches/Sanitizers); Lanes 15 – 19 represent Class C (Surface and Floor Mopping Cleaners/Detergents); and Lanes 20 – 22 represent Class D (Hand and Body Wash Gels). This comparative analysis allows descriptive visualization of the antimicrobial effectiveness relative to novobiocin, on one hand, and various classes (A – D), on the other hand, thereby showing the most effective class and/or detergent within a given category against a specific type of bacteria. The number of experimental observations is n = 3, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, as compared with either novobiocin or absolute MetOH. NI = No inhibition.

Figure 3. Depictive comparative assessment of the antimicrobial efficacy of various detergents against gram-positive *Enterococcus* Group D (*E. faecalis*) bacteria, as compared with novobiocin (30 µg). The zone of inhibition of novobiocin was set as a reference (lane 24; horizontal straight line), and that for absolute methanol (MetOH) is shown in lane 23, and all values of the zones of inhibition at undiluted concentrations of disinfectant/sterilizer/antiseptic were compared against those references (Lanes 23 and 24). Lanes 1 – 5 represent Class A (*Daily Mouthwash*); Lanes 6 – 14 represent Class B (*Toilet Bowl Cleaners/Bleaches/Sanitizers*); Lanes 15 – 19 represent Class C (*Surface and Floor Mopping Cleaners/Detergents*); and Lanes 20 – 22 represent Class D (*Hand and Body Wash Gels*). This comparative analysis allows descriptive visualization of the antimicrobial effectiveness relative to novobiocin, on one hand, and various classes (A – D), on the other hand, thereby showing the most effective class and/or detergent within a given category against a specific type of bacteria. The number of experimental observations is n = 3, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, as compared with either novobiocin or absolute MetOH. NI = No inhibition

Figure 4. Depictive comparative assessment of the antimicrobial efficacy of various detergents against gram-positive *Staphylococcus aureus* bacteria, as compared with novobiocin (30 µg). The zone of inhibition of novobiocin was set as a reference (lane 24; horizontal straight line), and that for absolute methanol (MetOH) is shown in lane 23, and all values of the zones of inhibition at undiluted concentrations of disinfectant/sterilizer/antiseptic were compared against those references (Lanes 23 and 24). Lanes 1 – 5 represent Class A (*Daily Mouthwash*); Lanes 6 – 14 represent Class B (*Toilet Bowl Cleaners/Bleaches/Sanitizers*); Lanes 15 – 19 represent Class C (*Surface and Floor Mopping Cleaners/Detergents*); and Lanes 20 – 22 represent Class D (*Hand and Body Wash Gels*). This comparative analysis allows descriptive visualization of the antimicrobial effectiveness relative to novobiocin, on one hand, and various classes (A – D), on the other hand, thereby showing the most effective class and/or detergent within a given category against a specific type of bacteria. The number of experimental observations is n = 3, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, as compared with either novobiocin or absolute MetOH. NI = No inhibition.

Comparative analytical assessment of the zones of inhibition of various classes (A-D) with reference to novobiocin (30 µg) depicts the efficacious impact of those antiseptics and disinfectants against pathogenic bacteria. The zones of inhibition of classes A-D for *Bacillus subtilis* is shown in Figure 2. Similarly, The zones of inhibition of classes A-D for *Enterococcus faecalis* is shown in Figure

3. The zones of inhibition of classes A-D for *Staphylococcus aureus* is shown in Figure 4. The zones of inhibition of classes A – D for *Streptococcus pyogenes* is shown in Figure 5. The zones of inhibition of classes A – D for *Streptococcus Group B* (*Streptococcus agalactiae*) is shown in Figure 6.

Figure 5. Depictive comparative assessment of the antimicrobial efficacy of various detergents against gram-positive *Streptococcus* Group A (*S. pyogenes*) bacteria, as compared with novobiocin (30 µg). The zone of inhibition of novobiocin was set as a reference (lane 24; horizontal straight line), and that for absolute methanol (MetOH) is shown in lane 23, and all values of the zones of inhibition at undiluted concentrations of disinfectant/sterilizer/antiseptic were compared against those references (Lanes 23 and 24). Lanes 1 – 5 represent Class A (*Daily Mouthwash*); Lanes 6 – 14 represent Class B (*Toilet Bowl Cleaners/Bleaches/Sanitizers*); Lanes 15 – 19 represent Class C (*Surface and Floor Mopping Cleaners/Detergents*); and Lanes 20 – 22 represent Class D (*Hand and Body Wash Gels*). This comparative analysis allows descriptive visualization of the antimicrobial effectiveness relative to novobiocin, on one hand, and various classes (A – D), on the other hand, thereby showing the most effective class and/or detergent within a given category against a specific type of bacteria. The number of experimental observations is $n = 3$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, as compared with either novobiocin or absolute MetOH. NI = No inhibition.

Figure 6. Depictive comparative assessment of the antimicrobial efficacy of various detergents against gram-positive *Streptococcus* Group B (*S. agalactiae*) bacteria, as compared with novobiocin (30 µg). The zone of inhibition of novobiocin was set as a reference (lane 24; horizontal straight line), and that for absolute methanol (MetOH) is shown in lane 23, and all values of the zones of inhibition at undiluted concentrations of disinfectant/sterilizer/antiseptic were compared against those references (Lanes 23 and 24). Lanes 1 – 5 represent Class A (*Daily Mouthwash*); Lanes 6 – 14 represent Class B (*Toilet Bowl Cleaners/Bleaches/Sanitizers*); Lanes 15 – 19 represent Class C (*Surface and Floor Mopping Cleaners/Detergents*); and Lanes 20 – 22 represent Class D (*Hand and Body Wash Gels*). This comparative analysis allows descriptive visualization of the antimicrobial effectiveness relative to novobiocin, on one hand, and various classes (A – D), on the other hand, thereby showing the most effective class and/or detergent within a given category against a specific type of bacteria. The number of experimental observations is $n = 3$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, as compared with either novobiocin or absolute MetOH. NI = No inhibition.

3.7. The Maximal Effective Ratios of Various Household Disinfectants

The putative immunomodulatory/anti-inflammatory, anti-microbial and bactericidal mechanisms are estimated by determining the probable effective ratios. The maximal effective ratio (E_R) of Classes A-D was calculated as the ratio of each bacterium with maximal zone of inhibition

against the minimum zone of inhibition (set as 1) within the same category, such that $E_R = \text{Zone}_{\max} / \text{Zone}_{\min}$. This ratio determines the most effective treatment for each bacterium and its comparative effectiveness against rest of antiseptics and disinfectants. The E_R of Class A is shown in Figure 7. The E_R of Class B is shown in Figure 8. The E_R of Class C is shown in Figure 9. The E_R of Class D is shown in Figure 10.

Figure 7. The putative immunomodulatory/anti-inflammatory, anti-microbial and bactericidal mechanisms are estimated by determining the probable effective ratios. The maximal effective ratio (E_R) of Class A (*Daily Mouthwash*) on gram-positive bacteria. E_R was calculated as the ratio of each bacterium with maximal zone of inhibition against the minimum zone of inhibition (set as 1) within the same category, such that $E_R = \text{Zone}_{\max} / \text{Zone}_{\min}$. This ratio determines the most effective treatment for each bacterium and its comparative effectiveness against rest of antiseptics and disinfectants. For instance, the highest most effective daily mouthwash against *S. aureus* is 'Colgate Plax Mouthwash.' The number of experimental observations is $n = 3$.

Figure 8. The putative immunomodulatory/anti-inflammatory, anti-microbial and bactericidal mechanisms are estimated by determining the probable effective ratios. The maximal effective ratio (E_R) of Class B (*Toilet Bowl Cleaners/Bleaches/Sanitizers*) on gram-positive bacteria. E_R was calculated as the ratio of each bacterium with maximal zone of inhibition against the minimum zone of inhibition (set as 1) within the same category, such that $E_R = \text{Zone}_{\max} / \text{Zone}_{\min}$. This ratio determines the most effective treatment for each bacterium and its comparative effectiveness against rest of antiseptics and disinfectants. For instance, the highest most effective daily mouthwash against *S. aureus* are 'Carrefour Nettoyant Disinfectant' and 'Harpic Power Plus Disinfectant.' The number of experimental observations is $n = 3$.

Figure 9. The putative immunomodulatory/anti-inflammatory, anti-microbial and bactericidal mechanisms are estimated by determining the probable effective ratios. The maximal effective ratio (E_R) of Class C (*Surface and Floor Mopping Cleaners/Detergents*) on gram-positive bacteria. E_R was calculated as the ratio of each bacterium with maximal zone of inhibition against the minimum zone of inhibition (set as 1) within the same category, such that $E_R = \text{Zone}_{\max} / \text{Zone}_{\min}$. This ratio determines the most effective treatment for each bacterium and its comparative effectiveness against rest of antiseptics and disinfectants. For instance, the highest most effective daily mouthwash against *S. aureus* is 'Ajax Fete des Fleurs.' The number of experimental observations is $n = 3$.

Class D – Hand and Body Wash Gels

Maximal Effective Ratio (E_R)

Figure 10. The putative immunomodulatory/anti-inflammatory, anti-microbial and bactericidal mechanisms are estimated by determining the probable effective ratios. The maximal effective ratio (E_R) of Class D (Hand and Body Wash Gels) on gram-positive bacteria. E_R was calculated as the ratio of each bacterium with maximal zone of inhibition against the minimum zone of inhibition (set as 1) within the same category, such that $E_R = \text{Zone}_{\max} / \text{Zone}_{\min}$. This ratio determines the most effective treatment for each bacterium and its comparative effectiveness against rest of antiseptics and disinfectants. For instance, the highest most effective daily mouthwash against *S. aureus* is 'HiGeen Hand and Body Wash Gel.' The number of experimental observations is $n = 3$.

Figure 11. Typical microbial growth of gram-positive bacteria in the presence of commercially available disinfectants and antiseptics in culture. (A) *Bacillus subtilis* + 'HiGeen Hand and Body Wash Gel' at various concentrations (undiluted, 1/2, 1/4, 1/8, 1/16, and 1/32 + negative control, methanol), noting zones of inhibition. (B) Group D *Streptococcus* (GDS; *E. faecalis*) + 'Carrefour Nettoyant Disinfectant' at various concentrations (undiluted, 1/2, 1/4, 1/8, 1/16, and 1/32 + positive control, novobiocin (30 µg)), noting zones of inhibition. (C) *Staphylococcus aureus* + 'Spartan Max WC Lavender' at various concentrations (undiluted, 1/2, 1/4, 1/8, 1/16, and 1/32 + positive control, novobiocin (30 µg)), noting zones of inhibition. (D) Group A *Streptococcus* (GAS) + 'La Croix Sans Javel' at various concentrations (undiluted, 1/2, 1/4, 1/8, 1/16, and 1/32 + negative control, methanol), noting zones of inhibition. (E) Group B *Streptococcus* (GBS) + 'Clorox Bleach Rain Clean' at various concentrations (undiluted, 1/2, 1/4, 1/8, 1/16, and 1/32 + positive control, novobiocin (30 µg)), noting zones of inhibition. The number of experimental observations is $n = 3$. DF = Dilution factor.

3.8. Typical Microbial Growth under the Influence of Selective Household Disinfectants

Typical microbial growth of gram-positive bacteria in the presence of commercially available disinfectants and antiseptics in culture is shown in Figure 11. The growth of *Bacillus subtilis* in the presence of 'HiGeen Hand and Body Wash Gel' at various concentrations (undiluted, 1/2, 1/4, 1/8, 1/16, and 1/32 + negative control, methanol), noting zones of inhibition is shown in Figure 11A. The growth of Group D *Enterococcus* (*E. faecalis*) in the presence of 'Carrefour Nettoyant Disinfectant' at various concentrations (undiluted, 1/2, 1/4, 1/8, 1/16, and 1/32 + positive control, novobiocin (30 µg)), noting zones of inhibition is shown in Figure 11B. The growth of *Staphylococcus aureus* in the presence of 'Spartan Max WC Lavender' at various concentrations (undiluted, 1/2, 1/4, 1/8, 1/16, and 1/32 + positive control, novobiocin (30 µg)), noting zones of inhibition is shown in Figure 11C. The growth of Group A *Streptococcus* (GAS) in the presence of 'La Croix Sans Javel' at various concentrations (undiluted, 1/2, 1/4, 1/8, 1/16, and 1/32 + negative control, methanol), noting zones of inhibition is shown in Figure 11D. The growth of Group B *Streptococcus* (GBS) in the presence of 'Clorox Bleach Rain Clean' at various concentrations (undiluted, 1/2, 1/4, 1/8, 1/16, and 1/32 + positive control, novobiocin (30 µg)), noting zones of inhibition is shown in Figure 11E.

4. Discussion

4.1. Infection Control and Microbial Analysis

The EPA has recently published a consortium on public health issues pertaining to disinfectants, sterilizers, and antiseptics that are commonly used by the public consumers [30-35]. According to the EPA, antimicrobials used in public healthcare settings are defined as 'substances that are used to destroy or suppress the growth of microorganisms [saprophytic or otherwise pathogenic], such as bacteria, viruses, or fungi that [may] pose a threat to humans [and their health welfare]'. The aforementioned consumer-targeted products are ostensibly effective in curbing the growth and/or spread of infectious microorganisms that are usually residing in or on non-living, inanimate surfaces, and on living tissues as well [36,37,38]. Of those commercially available products, sterilizers, disinfectants, and sanitizers are commonly known and widely used. Many of these products are anti-inflammatory in nature at sub-physiologic concentrations [1]; however, at supraphysiologic concentrations, they may exert inflammatory and/or irritant responses that may bear the imprints of allergic conditions [1,2,3,4,5].

4.2. Healthcare Products and Categorization

It is essentially pragmatic to consider what the differences among the various types of healthcare products actually are and how they are comparatively related to each other [39-50]. Firstly, *sterilizers* are considered products that are primarily designed to destroy microbes of myriad types including, but not limiting to,

fungi, viruses, and bacteria and their resilient spores. For instance, liquid sterilants are commonly used in medical settings essentially on selected delicate medical and surgical instruments, and equipment that cannot observably tolerate high temperature sterilization, where low temperature gas sterilization is usually not feasible [1,51,52,53,54,55]. Secondly, *disinfectants*, on the other hand, are healthcare products that are essentially used on inanimate surfaces and/or objects to control the growth of fungi, viruses, and bacteria; perhaps, spores are usually resistant to this kind of disinfectants as opposed to sterilizers [56-62]. The EPA has also categorized disinfectants a notch further, as follows: i) Hospital disinfectants (specific with a narrow activity spectrum); and ii) General use disinfectants (common household detergents with a broad activity spectrum). Moreover, there are four known types of commercially available disinfectants: 1) Chlorine-containing bleaches, a group of strong oxidizing agents comprising chlorine (e.g., *Perio.Kin Chlorhexidina*, *WC Net Bleach Gel*, *Carrefour Nettoyant Disinfectant*, *La Croix Sans Javel*, and *Clorox Bleach Rain Clean* used in this study); 2) Phenolic-containing compounds and detergents, derived from phenol, a caustic, poisonous, and white crystalline molecule (C_6H_5OH), commonly used in resins, disinfectants, plastics, and pharmaceuticals (e.g., *Spartan Septol Antiseptic/Disinfectant*, and *Astonish Vac Maxx* used in this study); 3) Pine oil-containing products, usually obtained by steam distillation processing of gum taken from pine trees, or chemically derived as a byproduct of paper pulp-making by a complicated sulfating process; and 4) Quaternary ammonium compounds (QACs) and detergents, essentially derived from ammonium cations (NH_4^+) to generate so often ammonium salts (e.g., *Mr. Muscle Toilet Cleaner Duck*, and *Germicidal Bowl Cleanse Spartan Flash* used in this study) [60-75]. Thirdly, *sanitizers* are recognized as products that tend to reduce, but not necessarily eliminate, microorganisms commonly found on inanimate objects. For example, sanitizing rinses are used for surfaces such as dishes and cooking utensils, equipment and utensils used in food-processing plants, and food service establishments [76-90]. This categorization of commercially available disinfecting and sanitizing detergents is significantly harnessing attention in terms of safe and healthy choices available to consumers in the current momentum of containing and curbing microbial infection and contamination [1].

4.3. Infection Control and Microbial Epidemiology

In healthcare settings, routine hygienic practices are mandatory and this certainly has assisted healthcare professionals in following standardized procedures to ensure quality infection control [1,2,3]. Recently, the 'Association for Professionals in Infection Control and Epidemiology (APIC)' [15], in a manner consistent with policies of EPA, has introduced strict infection control guidelines that have been integrated into a system of norms, especially at hospitals, in an attempt to ameliorate microbial resistance and/or spreading in many common setups [91-115]. Although household disinfectants and antiseptics are likely used at hospitals, specific considerations for healthcare settings demand the use of

clinically (and perhaps scientifically) proven effective disinfectants. The APIC has further published a series of definitions the authors recognize as 'necessary modules in curbing infection', and hence forward the reader's attention to commonly used definitions [1-5,116-125]:

A "Sterilization is the complete elimination or destruction of all forms of microbial life. It is accomplished in the hospital by either physical or chemical processes. Steam under pressure, dry heat, ethylene oxide gas, and liquid chemicals are the principle sterilizing agents used in the hospital."

B "Disinfection describes a process that eliminates many of all pathogenic microorganisms on inanimate objects with the exception of bacterial spores. This is generally accomplished by the use of liquid chemicals or wet pasteurization in health care settings. The efficacy of disinfection is affected by a number of factors; each of which may nullify or limit the efficacy of the process. Some of the factors that have been shown to affect disinfection efficacy are the prior cleaning of the object, the organic load on the object, the type and level of microbial contamination, the concentration of and exposure time to the germicide, the physical configuration of the object, and the temperature and pH of the disinfection process. The levels of disinfection are defined as sterilization, high-level disinfection, intermediate-level disinfection, and low-level

disinfection. High-level disinfection can be expected to destroy all microorganisms with the exception of high numbers of bacterial spores. Intermediate-level disinfection inactivates *Mycobacterium tuberculosis*, vegetative bacteria, most viruses and most fungi, but, does not necessarily kill bacterial spores. Low-level disinfection can kill most bacteria, some viruses and some fungi, but, cannot be relied onto kill resistant microorganisms or bacterial spores."

C "Cleaning is the removal of all foreign material (i.e., soil, organic material) from objects. It is normally accomplished with water, mechanical action, and detergents. Cleaning must precede disinfection and sterilization procedures."

D "Germicide is an agent that destroys microorganisms, particularly pathogenic organisms (germs)."

E "Chemical sterilants are chemicals used for the purpose of destroying all forms of microbial life, including fungal and bacterial spores."

F "Disinfectant is a germicide that inactivates virtually all recognized pathogenic microorganisms, but, not necessarily all microbial forms on inanimate objects."

G "Antiseptic is a chemical germicide formulated for use on skin or tissue and should not be used to decontaminate inanimate objects."

Table 23. The chemical composition of commercially available disinfectants and antiseptics according to their class of classification and antimicrobial mechanisms

Mechanisms, Active Chemical Composition, and Antimicrobial Action [§]		
Target	Disinfectant / Antiseptic	Mechanism (s) of Action
Cell envelope (cell wall, outer membrane)	Glutaraldehyde	Cross-linking of proteins
	EDTA, other permeabilizers	Gram-negative bacteria: Removal of Mg ²⁺ , release of some LPS
Cytoplasmic (inner) membrane	Quaternary ammonium compounds (QACs)	Generalized membrane damage involving phospholipid bilayers
	Chlorhexidine	Low concentrations affect membrane integrity, high concentrations cause congealing of cytoplasm
	Diamines	Induction of leakage of amino acids
	Polyhexanide (polyhexamethylene biguanide; PHMB), Alexidine	Phase separation and domain formation of membrane lipids
	Phenols	Leakage; some cause uncoupling
Cross-linking of macromolecules	Formaldehyde	Cross-linking of proteins, RNA, and DNA
	Glutaraldehyde	Cross-linking of proteins in cell envelope and elsewhere in the cell
DNA intercalation	Acridines	Intercalation of an acridine molecule between two layers of base pairs in DNA
Interaction with thiol groups	Silver compounds	Membrane-bound enzymes (interaction with thiol groups)
Effects on DNA	Halogens	Inhibition of DNA synthesis
	Hydrogen peroxide, silver ions	DNA strand breakage
Oxidizing agents	Halogens	Oxidation of thiol groups to disulfides, sulfoxides, or disulfonides
	Peroxygens	Hydrogen peroxide: Activity due to from formation of free hydroxy radicals (·OH), which oxidize thiol groups in enzymes and proteins; PAA: Disruption of thiol groups in proteins and enzymes

[§] Chemical composition of disinfectants and antiseptics is according to verified labels of purchased products. Commercially available consumer-targeted products were chosen to represent a range of products with respect to price and product type (encompassing both oxidative and non-oxidative biocides) and markets throughout the Middle East. (Adapted, courtesy of reference [33].)

Table 24. Standardized methods of sterilization and disinfection, according to APIC guidelines for infection control practice*

Sterilization			Disinfection		
<i>Critical items (will enter tissue or vascular system or blood will flow through them)</i>			<i>High-level (semi-critical items; will come in contact with mucous membrane or non-intact skin)</i>	<i>Intermediate-level (some semi-critical items and non-critical items)</i>	<i>Low-level (non-critical items; will come in contact with intact skin)</i>
Object	Procedure	Exposure Time (hr)	Procedure (exposure time ≥ 20 min) ^{b,c}	Procedure (exposure time ≤ 10 min)	Procedure (exposure time ≤ 10 min)
Smooth, hard surface ^a	A	MR	C	H	H
	B	MR	D	J	I
	C	MR	E	K	J
	D	6	F ^d		K
	E	6	G		L
Rubber tubing and catheters ^c	A	MR	C		
	B	MR	D		
	C	MR	E		
	D	6	F ^d		
	E	6			
Polyethylene tubing and catheters ^{c,e}	A	MR	C		
	B	MR	D		
	C	MR	E		
	D	6	F ^d		
	E	6			
Lensed instruments	B	MR	C		
	C	MR	D		
	D	6	E		
	E	6			
Thermometers (oral and rectal) ^f				H ^f	
Hinged instruments	A	MR	C		
	B	MR	D		
	C	MR	E		
	D	6			
	E	6			

* Modified from Rutala WA: In Wenzel, R.P.; ed. Prevention and control of nosocomial infections, Baltimore, 1987, Williams & Wilkins, pp. 257-282, and from Simmons, B.P. Am. J. Infect. Control., 1983, 11, 96-115.

A. Heat sterilization, including steam or hot air (see manufacturer's recommendations).

B. Ethylene oxide gas (see manufacturer's recommendations).

C. Glutaraldehyde-based formulations (2%). (A glutaraldehyde-phenate formulation at full strength also has been shown to sterilize items that are soaked for 6 ¾ hours. Caution should be exercised with all glutaraldehyde formulations when further in-use is anticipated.)

D. Demand-release chlorine dioxide (will corrode aluminum, copper, brass, series 400 stainless steel, and chrome, with prolonged exposure).

E. Stabilized hydrogen peroxide 6% (will corrode copper, zinc, and brass).

F. Wet pasteurization at 75°C for 30 minutes after detergent cleaning.

G. Sodium hypochlorite (1000 ppm available chlorine; will corrode metal instruments).

H. Ethyl or isopropyl alcohol (70% to 90%).

I. Sodium hypochlorite (100 ppm available chlorine).

J. Phenolic germicidal detergent solution (follow product label for use-dilution).

K. Iodophore germicidal detergent solution (follow product label for use-dilution).

L. Quaternary ammonium germicidal detergent solution (follow product label for use-dilution).

MR Manufacturer's recommendations.

^a See text for discussion.

^b The longer the exposure to a disinfectant, the more likely it is that all microorganisms will be eliminated. Ten minutes' exposure is not adequate to disinfect many objects, especially those that are difficult to clean, because they have narrow channels or other areas that can harbor organic material and bacteria. Twenty minutes exposure may be the minimum time needed to reliably kill M. tuberculosis with glutaraldehyde.

^c Tubing must be completely filled for disinfection; care must be taken to avoid entrapment of air bubbles during immersion.

^d Pasteurization (washer disinfectant) of respiratory therapy and anesthesia equipment is a recognized alternative to high-level disinfection. Some data challenge the efficacy of some pasteurization units.

^e Thermostability should be investigated when indicated.

^f Limited data suggest that at least 20 minutes exposure time is necessary. Do not mix rectal and oral thermometers at any stage of handling or processing.

4.4. Biochemical Analysis of Detergents and Disinfectants

The active chemical compositions of commercially available disinfectants and antiseptics according to their category of classification, showing the main active

component, recommended in-use concentration, supplier and trade name of the disinfectants used in this study are given in brevity in Table 23. The standardized methods of sterilization and disinfection, according to APIC guidelines for infection control practice are subsequently given in Table 24. This wide spectrum study has touched the very foundations of hygienic practice jibing with

internationally standardized procedures [126-140]. It certainly forms a unique approach to understanding the degree of infection control using commercially available disinfectants, antiseptic, and sterilants. Unaware of the humongous work at hand, we have though undertaken a daunting task of identifying commonly used disinfectants and antiseptics in the endeavor of creating public awareness and prowess consistent with established norms [1-5,141-155]. Therefore, the significance of this study falls in two parts: i) Identifying the efficacy and durability of household disinfectants in terms of controlling microbial growth; and ii) Providing a comparative canopy of information relevant to consumer's hygiene and public health awareness. Although we have not tackled the individual biochemical constituencies of the aforementioned household disinfectants, the stark variations in controlling the growth of gram-positive (and gram-negative) bacteria is in and of itself a daring process for taking the notion of infection control at home and farther afield safely and healthily another notch [156-162].

Comparatively, various disinfectants contain chemicals that are powerfully anti-bacterial (the certified labels attest to that, at least in theory). For example, household disinfectants are well known to contain chemicals such as aldehydes (R-CHO; usually non-corrosive, and stainless), alcohols (highly effective when this disinfectant is used on instruments, surfaces, and skin), hydrogen peroxide (H_2O_2), potassium permanganate ($KMnO_4$) solution, and iodine [163-175]. Moreover, disinfectants found in soaps and hand washes/sanitizers commonly contain phenol compounds, and their derivatives, which are highly effective anti-bacterial agents that have been consistently included in commercially available mouthrinse products as well, for example. On the other hand, antiseptics usually contain boric acid (H_3BO_3), alcohol, carbolic acid (C_6H_6O), iodine, H_2O_2 , sodium chloride (NaCl), calcium hypochlorite ($Ca(ClO)_2$), and chlorhexidine ($C_{22}H_{30}Cl_2N_{10}$). Interestingly, chlorine-containing products are as effective as bactericides, sporicides, and fungicides [175-183]. Furthermore, several factors might affect the degree of effectiveness of disinfectants and/or antiseptics. Those aspects that essentially determine antimicrobial efficacy are related to: i) Bacterial amount and concentration at the site being disinfected/sterilized; ii) The specific manner by which surfaces or objects or wounds are cleaned, especially if those sites are either flat or cracked; and iii) Dependency on variables such as blood stains, tissue or mucous, environmental temperature, exposure time, and chemical composition and stability, the latter being controlled by EPA [1,22,35,67,125,156,180-185]. In brevity, it is conspicuously understandable, therefore, that the effectiveness of disinfectants and/or antiseptics varies with cleanliness, exposure time, concentration, and temperature. Those not necessarily combined sequential modules are essentially crucial to determining the efficacy of commercially available household disinfectants, an issue that is significantly reflecting the pervasive nature of marketed antimicrobial products.

4.5. Antimicrobial Mechanisms of Detergents and Disinfectants

Analytically, this study has classified disinfectants and antiseptics into four main categories: i) Class A-Daily

mouthwash; ii) Class B-Toilet bowl cleaners/ bleaches/ sanitizers; iii) Class C – Surface and floor mopping cleaners/detergents; and iv) Class D – Hand and body wash gels. Those classes are by no means reflecting any degree of effectiveness, rather are a mirror of handy arrangement for chronological research purposes. Thereafter, we will map out a comparative analytical approach in simulating the descending order of antimicrobial efficacy of each class of disinfectant/sterilizer/ antiseptic used in this study against the individual gram-positive bacteria therein assessed [185-190]:

1. *Bacillus subtilis* – Class B > Class C > Class D > Class A;
2. *Enterococcus* Group D (*E. faecalis*) – Class B > Class A > Class C > Class D;
3. *Staphylococcus aureus* – Class B > Class A > Class C > Class D;
4. *Streptococcus* Group A (GAS; *S. pyogenes*) – Class B > Class C > Class A > Class D;
5. *Streptococcus* Group B (GBS; *S. agalactiae*) – Class B > Class A > Class D > Class C.

The first study that investigated the use of disinfectants at home was presented in 1978 [1]. Thereafter, an astronomical number of references, here in alluded to, investigated the antimicrobial disinfectants frequently used in hospitals, dental surgeries (and other healthcare settings), industry, and households. These disinfectants, as indicated above, include active ingredients such as alcohol (such as ethanol or isopropanol), which is usually wiped over inanimate surfaces (benches), and skin, and allowed to evaporate quickly; aldehyde (such as formaldehyde or glutaraldehyde), which is highly effective against bacteria; ammonia, which is usually added with chloramine, a disinfectant; chlorine, which usually reduces and/or neutralizes waterborne infectious agents; sodium hypochlorite, which is a common household bleach, highly effective disinfectant; H_2O_2 , effectively antibacterial and antiviral disinfectant; ozone, a gaseous disinfectant and highly effective antibacterial and antifungal sanitary disinfectant; phenol, which is common in most household detergents and in some daily mouthwash products, and is highly effective antiseptic; and quaternary ammonium salts (quats) (such as benzalkonium chloride), which are effectively antibacterial and act as biocides [190-201].

The wide canopy of household products investigated in the present study contained all of the abovementioned active ingredients, albeit with varying compositions and concentrations, many of which are antimicrobial. Via mapping the localities of bacteria, moreover, and scanning the milieu of common bacterial species in human mouth we have revealed families of gram-positive bacteria such as *Staphylococci*, *Streptococci*, *Lactobacilli*, a group of lactic acid-producing *Streptococci*, *Actinomyces*, and *Corynebacteria*, and gram-negative bacteria such as *Escherichia coli*, *Neisseria*, and *Fusobacteria*. According to recent reports, the most common household items that are likely to be infested with microbes are kitchen sponges and rags, dish towels, cutting boards, kitchen surfaces, sink drains, toilet, tub and shower, door knobs and handles, cellphones, computer keyboards, television remotes, carpets, and toothbrushes. Furthermore, common bacteria in household floors, bowels, lavenders, appliances, and

furniture are *Bacillus*, *Corynebacterium*, *Cryptosporidium*, *E. coli*, *Salmonella*, *Staphylococcus* spp., and *Streptococcus* spp. The majorities of the aforementioned bacteria are pathogenic in nature and have been examined in this pilot study. In terms of the highest efficacies (***) $P \leq 0.001$, the following is noticed: i) *Bacillus subtilis* – HiGeen Hand and Body Wash Gel > Carrefour Nettoyant Disinfectant > Vim Cream Multipurpose Fast Rinsing; ii) *Enterococcus faecalis* – Sensodyne Pronamel Mouthwash > WC Net Bleach Gel > Carrefour Nettoyant Disinfectant > Oral-B Pro-Expert Mouthwash; iii) *Staphylococcus aureus* – Colgate Plax Mouthwash = Carrefour Nettoyant Disinfectant = Harpic Power Plus Disinfectant = HiGeen Hand and Body Wash Gel; iv) *Streptococcus pyogenes* (GAS) – Dettol Antiseptic/Disinfectant > WC Net Bleach Gel > HiGeen Hand and Body Wash Gel; and v) *Streptococcus agalactiae* (GBS) – WC Net Bleach Gel > Carrefour Nettoyant Disinfectant > HiGeen Hand and Body Wash Gel > Sensodyne Pronamel Mouthwash [1,15-25,92-98,118-125,145-167].

4.6. Immunomodulatory/Inflammatory and Anti-inflammatory Putative Mechanisms of Detergents and Disinfectants

Antiseptics and disinfectants are used extensively in hospitals and other health care settings for a variety of topical and hard-surface applications. In particular, they are an essential part of infection control practices and aid in the prevention of nosocomial infections. Mounting concerns over the potential for microbial contamination and infection risks in the food chain and general consumer markets have also led to increased use of antiseptics and disinfectants by the general public [1,42]. A wide variety of active chemical agents (or “biocides”) are found in these products, many of which have been used for hundreds of years for antiseptics, disinfection, and preservation. Despite this, less is known about the mode of action of these active agents than about antibiotics. In general, biocides have a broader spectrum of activity than antibiotics, and, while antibiotics tend to have specific intracellular targets, biocides may have multiple targets. The widespread use of antiseptic and disinfectant products has prompted some speculation on the development of microbial resistance, in particular cross resistance to antibiotics [1,18,64,144]. Although the anti-microbial effects of commercially available detergents and disinfectants are now well established following the canopy of microorganisms investigated in this and other research studies, the inflammatory and/or anti-inflammatory mechanisms have yet to be unraveled [4,11,18,30,42,58,64,141,144,195,196]. Several hypotheses have been proposed as to deciphering the anti-microbial and inflammatory/anti-inflammatory effects of commercially available disinfectants and sterilizers, whose active ingredients in particular are essentially highly potent biocides. One of the scenarios indicated that the active ingredients of these detergents are irritants at certain concentrations and allergic reactions have been reported [42]. These inflammatory and allergic responses are ostensibly dependent on the frequency and time exposure, in addition to biochemical constituency and its variations. Furthermore, other scenarios implicated the occurrence of

anti-inflammatory effects in curbing the spread of microbial contamination in various healthcare settings, as alluded to above [11,18,42,58,141,195]. These opposing effects highlight the importance of understanding the mechanisms pertaining to infection control using those products. Current studies at our laboratories are investigating the purported anti-inflammatory effects at various levels: i) Measuring the minimum inhibitory concentrations (MICs) of various detergents against gram-positive and gram-negative bacteria *in vitro*; ii) Investigating the inflammatory and allergic responses at various concentrations, particularly that of hives and contact dermatitis; iii) Assessing the anti-inflammatory role of detergents and disinfectants commonly used in the dental office against gingivitis and plaques; iv) Undertaking the *in vivo* analytical assessment of the effect of detergents and disinfectants on inflammatory responses mediated by an essential transcription factor known as nuclear factor- κ B (NF- κ B); and v) Measuring cellular responses in terms of the effect of detergents and disinfectants on the biosynthesis and secretion of inflammatory cytokines *in vitro*. These observations jibe with the established efficacious role that detergents and disinfectants may exert both anti-microbial and anti-inflammatory effects *in vitro* and *in vivo* [1,42].

Considerable progress has been made in understanding the mechanisms of the antibacterial action of antiseptics and disinfectants. By contrast, studies on their modes of action against fungi, viruses, and protozoa have been rather sparse. Furthermore, little is known about the means whereby these agents inactivate prions [1]. Whatever the type of microbial cell (or entity), it is probable that there is a common sequence of events. This can be envisaged as interaction of the antiseptic or disinfectant with the cell surface followed by penetration into the cell and action at the target site(s). The nature and composition of the surface vary from one cell type (or entity) to another but can also alter as a result of changes in the environment. Interaction at the cell surface can produce a significant effect on viability (e.g. with glutaraldehyde), but most antimicrobial agents appear to be active intracellularly [1]. The outer most layers of microbial cells can thus have a significant effect on their susceptibility (or insusceptibility) to antiseptics and disinfectants; it is disappointing how little is known about the passage of these antimicrobial agents into different types of microorganisms. A battery of techniques are currently available for studying the mechanisms of action of antiseptics and disinfectants on microorganisms, especially bacteria [1,42,55,65,112,145,196]. These include the examination of uptake, lysis and leakage of intracellular constituents, perturbation of cell homeostasis, effects on model membranes, inhibition of enzymes, electron transport, and oxidative phosphorylation, interaction with macromolecules, effects on macromolecular biosynthetic processes, and microscopic examination of biocide-exposed cells. Additional and useful information can be obtained by calculating concentration exponents (n values) and relating these to membrane activity. Many of these procedures are valuable for detecting and evaluating antiseptics or disinfectants used in combination [1]. Antimicrobial and bactericidal activity of typical commercial disinfectants are given in Table 25.

Table 25. Antimicrobial and bactericidal activity of typical commercial disinfectants*

Disinfectant Type	Gram-Positive Bacteria	Gram-Negative Bacteria	Bacterial Spores	Viruses	Fungi
Sodium hypochlorite and other chlorine compounds	+++	+++	++	++	++
Iodine	+++	+++	+++	++	++
Iodophors	+++	+++	+	++	++
Black and white fluids	+++	+++	+	+	+
Chloroxynol	+++	+	–	N.A.	N.A.
Pine oil	+	+	–	N.A.	N.A.
Chlorhexidine	+++	++	–	+	+
Cetrimide	+++	++	–	+	+

* +++, High antimicrobial; ++, Moderate antimicrobial; +, Weak antimicrobial; –, Not antimicrobial; N.A., Not applicable. Adapted, courtesy of reference [1].

4.7. Standards for Performance of Disinfectants and Sterilants

According to the ‘Therapeutic Goods Act 1989’ of the Australian ‘Department of Health and Ageing’, there are specific standards that should be recognized for optimum performance of disinfectants and sterilants.

I. Where the directions for use on a label attached to or appearing on the container or a primary pack of a therapeutic good represent it to be a *hospital grade disinfectant*, the minimum performance requirement shall apply as follows:

- A. Where the disinfectant is for general purpose use on surfaces:
 - a. If tested in accordance with the prescribed test it must, pass the prescribed test under the conditions specified in the prescribed test; and
 - b. Pass a suitable bactericidal carrier test; and
 - c. Pass suitable sporicidal, fungicidal, tuberculocidal, virucidal or other biocidal tests only where a claim is made in respect of any of these actions; and
 - d. Pass each of the tests in (i), (ii), and (iii) according to the conditions specified or claimed on the label, if any.
- B. When for use as a surface spray it must:
 - a. Pass a suitable bactericidal carrier test; and
 - b. Pass suitable sporicidal, fungicidal, tuberculocidal, virucidal or other biocidal tests only where a claim is made in respect of any of these actions; and
 - c. Pass each of the tests in (i) and (ii) according to the conditions specified or claimed on the label.
- C. Where the disinfectant is presented as a cloth wipe impregnated with disinfectant, and intended for single use or multiple use for disinfection of surfaces, it must:
 - a. If tested in accordance with the prescribed test, pass the prescribed test under the conditions specified in Option A or Option B of the prescribed test, when the test is carried out on the product after extraction from the wipe; and
 - b. Pass a suitable (single or multiple use) simulated in-use test; and

c. Pass suitable sporicidal, fungicidal, tuberculocidal, virucidal or other biocidal tests only where a claim is made in respect of any of these actions; and

d. Pass each of the tests in (i), (ii), and (iii) according to the conditions specified or claimed on the label, if any.

II. Where the directions for use on a label attached to or appearing on the container or primary pack of a therapeutic good represent it to be a *household/commercial grade disinfectant*, the minimum performance requirement shall apply as follows:

- A. Where the disinfectant is for general purpose use:
 - a. If tested in accordance with the prescribed test it must, pass the prescribed test under the conditions specified in the prescribed test; or
 - b. Pass a suitable bactericidal carrier test; and
 - c. Pass suitable sporicidal, fungicidal, tuberculocidal, virucidal or other biocidal tests only where a claim is made in respect of these actions; and
 - d. Pass each of the tests in (i), (ii) and (iii) according to the conditions specified or claimed on the label, if any.
- B. Where the disinfectant is for use as a surface spray it must:
 - a. Pass a suitable bactericidal carrier test; and
 - b. Pass suitable sporicidal, fungicidal, tuberculocidal, virucidal or other biocidal tests only where a claim is made in respect of any of these actions; and
 - c. Pass each of the tests in (i) and (ii) according to the conditions specified or claimed on the label.
- C. Where the disinfectant is presented as a cloth wipe impregnated with disinfectant and intended for single use or multiple use on surfaces, it must:
 - a. If tested in accordance with the prescribed test, pass the prescribed test under the conditions specified in the prescribed test, when the test is carried out on the product after extraction from the wipe; and
 - b. Pass a suitable (single or multiple) simulated in-use test; and

- c. Pass suitable sporicidal, fungicidal, tuberculocidal, virucidal or other biocidal tests only where a claim has been made in respect of any of these actions; and
- d. Pass each of the tests in (i), (ii) and (iii) according to the conditions specified or claimed on the label, if any.

III Where different uses for a disinfectant are specified in a label on the container or primary pack containing the disinfectant and different conditions are recommended on the label for each use, each label claim should meet the prescribed test for that type of use. The test should be carried out at the lowest concentration of disinfectant recommended on the label (where the product is to be diluted before use) for that use and at the end of the shelf life of the disinfectant prepared for that use.

IV Notwithstanding the requirements set forth, a disinfectant shall not be regarded as having failed to pass the prescribed test unless:

- A. It fails to pass the prescribed test on each occasion of the 3 occasions of testing; or
- B. Where it fails to pass the prescribed test on 1 or 2 of the 3 occasions of testing and the prescribed test is again carried out, it fails to pass the prescribed test when it again fails any of the three occasions of the test.

V Microbial stability testing must be conducted on the final formulation, which must pass the most stringent suitable test in accordance with Clause 3 for the grade of disinfectant at the end of the real time shelf life. Microbial stability studies must be repeated to confirm shelf life whenever there are changes to the manufacturing process, the formula or the packaging and when the shelf life is to be extended.

VI In relation to the microbial quality of disinfectants, ideally, there should be no microbial contamination.

However, unless products are intended to be sterile, it is likely that some contamination may be present. This should be kept to a minimum and must not contain pathogenic organisms or inappropriate organisms (i.e., vegetative bacteria in a product marketed as bactericidal).

Figure 12. Descending order of resistance to antiseptics and disinfectants. This map may resemble a variation of an order in that the conclusions are not yet universally agreed upon. (Adapted, courtesy of reference [33])

Type of resistance	Example(s)	Mechanism of resistance
Impermeability		
Gram-negative bacteria	QACs, triclosan, diamines	Barrier presented by outer membrane may prevent uptake of antiseptic or disinfectant; glycocalyx may also be involved
Mycobacteria	Chlorhexidine, QACs Glutaraldehyde	Waxy cell wall prevents adequate biocide entry Reason for high resistance of some strains of <i>M. chelonae</i> (?)
Bacterial spores	Chlorhexidine, QACs, phenolics	Spore coat(s) and cortex present a barrier to entry of antiseptics and disinfectants
Gram-positive bacteria	Chlorhexidine	Glycocalyx/mucoexopolysaccharide may be associated with reduced diffusion of antiseptic
Inactivation (chromosomally mediated)	Chlorhexidine	Breakdown of chlorhexidine molecule may be responsible for resistance

Figure 13. Bacterial intrinsic resistance mechanisms to antiseptics and disinfectants. (Adapted, courtesy of reference [33])

4.8. Detergents and Disinfectants Recap at a Glance

In recapitulation, the wide spectrum of household products carefully used therein has shown interesting

antimicrobial variations in terms of efficacies [1]. Regarding Class A (*Daily Mouthwash*), the most effective against *Bacillus subtilis* is 'ColgatePlax Mouthwash'; the most effective against *Enterococcus faecalis* are 'Sensodyne Pronamel Mouthwash' and 'Oral-B Pro-

Expert Mouthwash' (almost equal occurrences); the most effective against *Staphylococcus aureus* is 'Colgate Plax Mouthwash'; the most effective against *Streptococcus pyogenes* is 'Colgate Plax Mouthwash'; and the most effective against *Streptococcus agalactiae* is 'Sensodyne Pronamel Mouthwash'. Regarding Class B (*Toilet Bowl Cleaners/Bleaches/Sanitizers*), the most effective against *Bacillus subtilis* is 'Carrefour Nettoyant Disinfectant'; the most effective against *Enterococcus faecalis* are 'WC Net Bleach Gel' and 'Carrefour Nettoyant Disinfectant' (almost equal occurrences); the most effective against *Staphylococcus aureus* are 'Carrefour Nettoyant Disinfectant' and 'Harpic Power Plus Disinfectant' (almost equal occurrences); the most effective against *Streptococcus pyogenes* is 'WC Net Bleach Gel'; and the most effective against *Streptococcus agalactiae* is 'WC Net Bleach Gel'. Regarding Class C (*Surface and Floor Mopping Cleaners/Detergents*), the most effective against *Bacillus subtilis* is 'Vim Cream Multipurpose Fast Rinsing'; the most effective against *Enterococcus faecalis* are 'Dettol Antiseptic/Disinfectant' and 'Spartan Septol Antiseptic/Disinfectant' (almost equal occurrences); the most effective against *Staphylococcus aureus* is 'Ajax

Fete des Fleurs'; the most effective against *Streptococcus pyogenes* is 'Dettol Antiseptic/Disinfectant'; and the most effective against *Streptococcus agalactiae* is 'Dettol Antiseptic/Disinfectant'. Regarding Class D (*Hand and Body Wash Gels*), the most effective against *Bacillus subtilis* is 'HiGeen Hand and Body Wash Gel'; the most effective against *Enterococcus faecalis* is 'HiGeen Hand and Body Wash Gel'; the most effective against *Staphylococcus aureus* is 'HiGeen Hand and Body Wash Gel'; the most effective against *Streptococcus pyogenes* is 'HiGeen Hand and Body Wash Gel'; and similarly the most effective against *Streptococcus agalactiae* is 'HiGeen Hand and Body Wash Gel'. Descending order of resistance to antiseptics and disinfectants is shown in [Figure 12](#). Bacterial intrinsic resistance mechanisms to antiseptics and disinfectants is shown in [Figure 13](#). Typical examples on the effect of disinfectants containing chlorine on *Bacillus mycoides* is shown in [Figure 14](#). The effect of EO (essential oil rinse), CHX (0.12% chlorhexidine rinse), CPC/CHX (0.05% cetyl pyridinium chloriderinse/CHX), AFSF (amine fluoride/stannous fluoride rinse), and CPC2 (0.07% cetyl pyridinium chloride rinse) mouthrinses on saliva-derived biofilms is shown in [Figure 15](#).

Figure 14. Typical examples on the effect of disinfectants containing chlorine on *Bacillus mycoides*. (A) Acridine orange staining of *Bacillus mycoides* in sterile saline buffer (without chlorine) observed by epifluorescence microscopy. (B) Acridine orange staining of *Bacillus mycoides* in sterile saline buffer (after 60 min. of contact with 1 mg/L of chlorine) observed by epifluorescence microscopy. (Adapted, courtesy of reference [19])

Figure 15. Typical examples on the effects of EO (essential oil rinse), CHX (0.12% chlorhexidine rinse), CPC/CHX (0.05% cetyl pyridinium chloriderinse/CHX), AFSF (amine fluoride/stannous fluoride rinse), and CPC2 (0.07% cetyl pyridinium chloride rinse) mouthrinses on saliva-derived biofilms (two 30-second treatments, vital staining, batch biofilm model). *For abbreviations used in this illustration, please refer to abbreviations list.* (Adapted, courtesy of reference [113])

5. Conclusions and Prospects

The present wide spectrum study has meticulously examined the ostensible immunomodulatory antimicrobial efficacies of various household antiseptics and disinfectants to a surprising revelation of four classes, dubbed A-D. Whilst these commercially available products show variations in antimicrobial effectiveness, this is the first broad investigation that determined authenticity of information commercially inundating the public in terms of hygiene and health awareness [1]. For the first time in recent history that a study of this magnitude has ever been attempted. That said, we have not only revealed putative antimicrobial variations with myriad household products, but also unraveled the effectiveness of these products as compared with commonly used antibiotic, novobiocin, albeit showing in many occasions more antimicrobial propensity than the antibiotic itself. These results bolster the common observations that commercially available household products are effective antimicrobials at various levels, but that professional advertising is less than accurate and consumer's attention should be revisited and redirected. The choice of any of those products as common commodities essentially remains that of the consumer [1-5,25-30,45-62,91-105,116-132,174-182,197-220]. This study, nevertheless, has mirrored an unprecedented household guide roadmap for well-informed, prowess, and aware public health decisions relevant to hygiene, disinfection, sanitization, and infection control.

Conflict of Interest

The authors confirm that this article content has no conflicts of interest.

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Patient consent

The authors confirm that there is no patient consent involved with the bearings of this work.

Footnote

The authors would want to mention that this work therein reported and any other ramifications of this research thereafter demonstrated is and are not to be construed as an attempt to undermine or damage the integrity of information and/or validity of biochemical efficacies provided and promoted by commercial tenders or trademarks. We are consumers reporting observations that have been validated in a recognized research

laboratory, and hence we have no intention otherwise to disqualify or discredit any domestic or international brand or trademark. Therefore, the authors and or their institution thereby bear and hold no liability or any legal responsibility as we have reported original research work performed by students and their qualified instructors for educational purposes, and is not intended in any way, shape, or form to be viewed and/or construed for promotional or commercial endpoints.

Abbreviations

Aldehyde, R-CHO; American Chemical Society, ACS; Amine fluoride/stannous fluoride, AFSF; Association for Professionals in Infection Control and Epidemiology, APIC; Boric acid, H_3BO_3 ; Carboic acid, C_6H_6O ; Calcium hypochlorite, $Ca(ClO)_2$; Centers for Disease Control and Prevention, CDC; Cetyl pyridinium chloride, CPC; Chlorhexidine, $C_{22}H_{30}Cl_2N_{10}$ (CHX); Enterococcus Group D, EGD; Environmental Protection Agency, EPA; Essential oil, EO; Ethanol, EtOH; Group A *Streptococcus*, GAS; Group B *Streptococcus*, GBS; Hydrogen peroxide, H_2O_2 ; Minimum inhibitory concentration, MIC; Nuclear factor- κ B, NF- κ B; One-way analysis of variance, ANOVA; Phenol, C_6H_5OH ; Phosphate buffered saline, PBS; Potassium permanganate, $KMnO_4$; Quaternary ammonium compounds, QACs; Sodium chloride, NaCl; World Health Organization, WHO.

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