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

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Abstract **Background:** Immunomodulatory/anti-inflammatory and microbial infection control strategies characterize the spiral evolution of public awareness of health safety issues. This is substantiated with burgeoning number of cases of microbial contamination and/or infection in myriad healthcare settings, at the hospital, and even at home. Previously, we have investigated and identified laboratory parameters in the assessment of the antimicrobial effects of a myriad of commercial disinfectants on the growth of pathogenic and saprophytic gram-positive bacteria. The present sequel study investigates the antimicrobial/bactericidal effects of commercially available disinfectants, sterilizers, antiseptics, and chlorhexidine-containing detergents on the growth of saprophytic and pathogenic gram-negative 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 *Citrobacter koseri* is ‘Colgate Plax Mouthwash’; *Enterobacter cloacae* is ‘Colgate Plax Mouthwash’; *Escherichia coli* is ‘Colgate Plax Mouthwash’; *Escherichia coli* ESBL is ‘Colgate Plax Mouthwash’; *Klebsiella pneumoniae* is ‘Colgate Plax Mouthwash’; *Proteus vulgaris* is ‘Colgate Plax Mouthwash’; *Pseudomonas aeruginosa* is ‘Perio.Kin Chlorhexidina 0.20 %’; *Salmonella typhimurium* is ‘Colgate Plax Mouthwash’; and *Shigella sonnei* is ‘Colgate Plax Mouthwash’. Regarding Class B, the most effective against *C. koseri* is ‘Harpic Power Plus Disinfectant’; *E. cloacae* is ‘WC Net Bleach Gel’; *E. coli* is ‘WC Net Bleach Gel’; *E. coli* ESBL is ‘WC Net Bleach Gel’; *K. pneumoniae* are ‘WC Net Bleach Gel’ and ‘Harpic Power Plus Disinfectant’; *P. vulgaris* is ‘Spartan Max WC Lavender’; *P. aeruginosa* is ‘WC Net Bleach Gel’; *S. typhimurium* is ‘Clorox Bleach Rain Clean’; and *S. sonnei* is ‘Harpic Power Plus Disinfectant’. Regarding Class C, the most effective against *C. koseri* is ‘Dettol Antiseptic/Disinfectant’; *E. cloacae* is ‘Dettol Antiseptic/Disinfectant’; *E. coli* is ‘Vim Cream Multipurpose Fast Rinsing’; *E. coli* ESBL is ‘Dettol Antiseptic/Disinfectant’; *K. pneumoniae* is ‘Dettol Antiseptic/Disinfectant’; *P. vulgaris* is ‘Dettol Antiseptic/Disinfectant’; *P. aeruginosa* is ‘Dettol Antiseptic/Disinfectant’; *S. typhimurium* is ‘Dettol Antiseptic/Disinfectant’; and *S. sonnei* is ‘Dettol Antiseptic/Disinfectant’. Regarding Class D, the most effective against *C. koseri*, *E. cloacae, E. coli, E. coli* ESBL, *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa*, *S. typhimurium*, and *S. sonnei* is unprecedentedly the ‘HiGeen Hand and Body Wash Gel’. **Conclusions:** These laboratory results emphatically confirm and verify immunomodulatory infection control variations in the antimicrobial/anti-inflammatory effectiveness of household antiseptics and disinfectants that are purportedly identified in ameliorating the growth of saprophytic and pathogenic gram-negative bacteria in culture.

Keywords: *antimicrobial, anti-inflammation, antiseptics, bactericidal, biomedical laboratory, bleaches, ceftazidime, contamination, disinfectants, disk diffusion, gram-negative bacteria, household detergents, immunomodulation, infection control, sterilizers*

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1. Introduction

The behavioral obsession with infection control using commercially available disinfectants has inundated our way of living since the very dawn of modern society, as we know it [1,2]. Retrospectively, there has been a persistent accumulation of interest in the underlying causes of many house- and hospital-borne microbial-associated illnesses and disorders [3,4,5]. Subsequently, the market shelves have been spirally flooded with antimicrobial household products that have been incessantly introduced to have the ostensible ability of curbing bacterial infections and contaminations; that is certainly recognized an attempt to evaluate and measure the pervasiveness and effectiveness of the processes involved with infection control in public healthcare settings, points of care, households, and clinics [6-8]. 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]*."

Previously, we have examined the immunomodulatory/antimicrobial effects of a myriad of household detergents and disinfectants on the growth of saprophytic and pathogenic gram-positive bacteria [1]. Many attempts have been undertaken to quantitate the antimicrobial activities of household detergents. To the best that the authors know of, none of the aforementioned investigations has offered a wide canopy of analytical measurements on the 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 [1]*.

Considering the influence that the concept of infection control bears in our society today, this study is a pioneering attempt in determining the antimicrobial effect of virtually most of the commercially available disinfectants and antiseptics available in the market [9-15]. The study 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 [1,2]. 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 of humans in the very safety of their homes, and presents to the eager and perhaps unknowing consumers calculated, precise and definitive scientifically based choices for safe and healthy disinfectant selections, substantiated and corroborated with verified and validated laboratory analytical assessment [16-24].

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) [1].

2.2. Preparatory Methods and Design

2.2.1. Bacterial Strains

All bacterial strains studied in this report were gram-negative and included: Gram-negative rods (bacilli) – *Citrobacter koseri* (*C. koseri* – facultative anaerobe); *Enterobacter cloacae* (*E. cloacae* – facultative anaerobe); *Escherichia coli* (*E. coli* – facultative anaerobe); *Escherichia coli* ESBL (*E. coli* ESBL – facultative anaerobe); *Klebsiella pneumoniae* (*K. pneumoniae* – facultative anaerobe); *Proteus vulgaris* (*P. vulgaris* – facultative anaerobe); *Salmonella typhimurium* (*S. typhimurium* – facultative anaerobe); and *Shigella sonnei* (*S. sonnei* – facultative anaerobe); and Gram-negative coccobacilli – *Pseudomonas aeruginosa* (*P. aeruginosa* – aerobic). All clinical bacterial specimens that were properly collected and stored were gratis of the Clinical Laboratory Medicine departments at Hammoud Hospital University Medical Center (HH-UMC; Saida, Lebanon), and Al-Makassed General Hospital University Medical Center (MGH-UMC; Beirut, Lebanon) [1].

2.2.2. Disk Diffusion Method

Prior to experimental use, all bacterial strains were cultured, grown and maintained on nutrient agar medium, as previously described [1]. The widely used Muller-Hinton plates were seeded with bacterial inoculums (5 x 10^8 CFU/ml) [1,2,3,4,5]. 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 [6-12].

2.3. Statistical Analysis and Data Handling

Statistical analysis of the results was completed using Microsoft Office Excel 2013, as previously indicated [1]. Experimental results were expressed as mean ± 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 [1].

3.1. The Zones of Inhibition of Gram-Negative Bacterium *Citrobacter koseri*

3.1.1. The Zones of Inhibition of Class A

The effect of daily mouthwash (category Class A) on the microbial growth of *Citrobacter koseri* is given in Table 1 – Table 5. It is noted that 'Colgate Plax Mouthwash' is most effective in category Class A. The inhibitory effect of the commonly used antibiotic ceftazidime (30 μg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

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

Microorganism	Inhibition Zone Diameter (mm) [a]								
	control ddH_20	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (30 μg)
class A – Daily Mouthwash [§] Sensodyne Pronamel Mouthwash (Disinfectant) Active ingredients – Sodium fluoride (0.05%).									
Gram-Negative Bacteria									
Citrobacter koseri	NI *	NI	–	–	–	–	–	–	19.00 ± 0.18
Enterobacter cloacae	NI	NI	–	–	–	–	–	–	19.00 ± 0.15
Escherichia coli	NI	NI	–	–	–	–	–	–	15.00 ± 0.15
E. coli ESBL	NI	NI	–	–	–	–	–	–	0.00 ± 0.00
Klebsiella pneumoniae	NI	NI	–	–	–	–	–	–	25.00 ± 0.25
Proteus vulgaris	NI	NI	2.67 ± 1.54	–	–	–	–	–	25.00 ± 0.25
Pseudomonas aeruginosa	NI	NI	–	–	–	–	–	–	14.00 ± 0.12
Salmonella typhimurium	NI	NI	–	–	–	–	–	–	18.00 ± 0.18
Shigella sonnei	NI	NI	13.00 ± 0.33	7.67 ± 2.21	–	–	–	–	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 2. The inhibition zone diameter methodological analysis of the effect of daily mouthwash (class A) on the growth of gram-negative bacteria.

Microorganism	Inhibition Zone Diameter (mm) [a]								
	control ddH_20	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (30 μg)
class A – Daily Mouthwash [§] Oral-B Pro-Expert Mouthwash (Disinfectant) Active ingredients – Alcohol, Propyl paraben, and Poloxamer 407.									
Gram-Negative Bacteria									
Citrobacter koseri	NI *	NI	–	–	–	–	–	–	19.00 ± 0.18
Enterobacter cloacae	NI	NI	–	–	–	–	–	–	19.00 ± 0.15
Escherichia coli	NI	NI	–	–	–	–	–	–	15.00 ± 0.15
E. coli ESBL	NI	NI	–	–	–	–	–	–	0.00 ± 0.00
Klebsiella pneumoniae	NI	NI	–	–	–	–	–	–	25.00 ± 0.25
Proteus vulgaris	NI	NI	–	–	–	–	–	–	25.00 ± 0.25
Pseudomonas aeruginosa	NI	NI	–	–	–	–	–	–	14.00 ± 0.12
Salmonella typhimurium	NI	NI	–	–	–	–	–	–	18.00 ± 0.18
Shigella sonnei	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 3. The inhibition zone diameter methodological analysis of the effect of daily mouthwash (class A) on the growth of gram-negative bacteria

Microorganism	Inhibition Zone Diameter (mm) [a]								
	control ddH_20	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (30 µg)
class A – Daily Mouthwash [§] Colgate Plax Mouthwash (Disinfectant) Active ingredients – Cetylpyridinium chloride (0.05%, w/w), and Ethanol (7.3%).									
Gram-Negative Bacteria									
Citrobacter koseri	NI *	NI	19.00 ± 0.33	17.00 ± 0.32	10.67 ± 1.01	2.33 ± 1.34	–	–	19.00 ± 0.18
Enterobacter cloacae	NI	NI	29.33 ± 3.56	20.67 ± 1.07	17.33 ± 1.68	8.00 ± 2.40	6.33 ± 1.84	–	19.00 ± 0.15
Escherichia coli	NI	NI	29.33 ± 0.51	25.00 ± 0.33	21.33 ± 0.51	15.33 ± 0.51	6.67 ± 1.95	–	15.00 ± 0.15
E. coli ESBL	NI	NI	21.67 ± 0.19	18.33 ± 0.51	10.67 ± 0.52	9.00 ± 0.15	8.00 ± 0.12	2.32 ± 1.35	0.00 ± 0.00
Klebsiella pneumoniae	NI	NI	27.67 ± 0.19	21.33 ± 0.19	17.33 ± 0.69	13.67 ± 1.01	8.67 ± 0.19	–	25.00 ± 0.25
Proteus vulgaris	NI	NI	25.33 ± 2.36	14.00 ± 1.20	–	–	–	–	25.00 ± 0.25
Pseudomonas aeruginosa	NI	NI	–	–	–	–	–	–	14.00 ± 0.12
Salmonella typhimurium	NI	NI	31.00 ± 0.58	23.67 ± 2.17	17.33 ± 2.50	13.67 ± 1.07	5.00 ± 2.87	–	18.00 ± 0.18
Shigella sonnei	NI	NI	23.33 ± 0.51	19.67 ± 0.19	15.33 ± 0.51	9.68 ± 0.38	6.00 ± 1.73	5.33 ± 1.54	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 4. The inhibition zone diameter methodological analysis of the effect of daily mouthwash (class A) on the growth of gram-negative bacteria

Microorganis	Inhibition Zone Diameter (mm) [a]								
	control ddH_20	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (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-Negative Bacteria									
Citrobacter koseri	NI *	NI	–	–	–	–	–	–	19.00± 0.18
Enterobacter cloacae	NI	NI	–	–	–	–	–	–	19.00± 0.15
Escherichia coli	NI	NI	5.33± 1.57	–	–	–	–	–	15.00± 0.15
E. coli ESBL	NI	NI	–	–	–	–	–	–	0.00± 0.00
Klebsiella pneumoniae	NI	NI	–	–	–	–	–	–	25.00± 0.25
Proteus vulgaris	NI	NI	–	–	–	–	–	–	25.00± 0.25
Pseudomonas aeruginosa	NI	NI	–	–	–	–	–	–	14.00± 0.12
Salmonella typhimurium	NI	NI	–	–	–	–	–	–	18.00± 0.18
Shigella sonnei	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.

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 *Citrobacter koseri* is given in Table 6 – Table 14. It is noted that 'Harpic Power Plus Disinfectant' is most effective in category Class B. The inhibitory effect of the commonly used antibiotic ceftazidime (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 *Citrobacter koseri* is given in Table 15 – Table 19. It is noted that 'Dettol Antiseptic/Disinfectant' is most effective in category Class C. The inhibitory effect of the commonly used antibiotic ceftazidime (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 *Citrobacter koseri* is given in Table 20 – Table 22. It is noted that 'HiGeen Hand and Body Wash Gel' is most effective in category Class D. The inhibitory effect of the commonly used antibiotic ceftazidime (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-Negative Bacterium *Enterobacter cloacae*

3.2.1. The Zones of Inhibition of Class A

The effect of daily mouthwash (category Class A) on the microbial growth of *Enterobacter cloacae* is given in Table 1 – Table 5. It is noted that 'Colgate Plax Mouthwash' is most effective in category Class A. The inhibitory effect of the commonly used antibiotic ceftazidime (30 μg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

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

Microorganism	Inhibition Zone Diameter (mm) [a]								
	control $ddH_2 0$	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (30 μg)
class A – Daily Mouthwash [§] Perio.Kin Chlorhexidina 0.20% (Disinfectant) Active ingredients – Chlorhexidine (0.20%).									
Gram-Negative Bacteria									
Citrobacter koseri	NI *	NI	–	–	–	–	–	–	19.00 ± 0.18
Enterobacter cloacae	NI	NI	8.65 ± 0.19	–	–	–	–	–	19.00 ± 0.15
Escherichia coli	NI	NI	11.67 ± 0.52	12.33 ± 0.96	6.00 ± 1.73	2.33 ± 1.34	–	–	15.00 ± 0.15
E. coli ESBL	NI	NI	12.00 ± 0.33	10.33 ± 0.19	9.00 ± 0.12	9.00 ± 0.15	2.67 ± 1.53	–	0.00 ± 0.00
Klebsiella pneumoniae	NI	NI	–	–	–	–	–	–	25.00 ± 0.25
Proteus vulgaris	NI	NI	–	–	–	–	–	–	25.00 ± 0.25
Pseudomonas aeruginosa	NI	NI	8.32 ± 0.19	2.33 ± 1.34	–	–	–	–	14.00 ± 0.12
Salmonella typhimurium	NI	NI	8.00 ± 0.57	–	–	–	–	–	18.00 ± 0.18
Shigella sonnei	NI	NI	12.00 ± 0.08	10.33 ± 0.19	6.67 ± 1.92	5.33 ± 1.54	–	–	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.

3.2.2. The Zones of Inhibition of Class B

The effect of toilet bowl cleaners/bleaches/sanitizers (category Class B) on the microbial growth of *Enterobacter cloacae* is given in Table 6 – Table 14. It is noted that 'WC Net Bleach Gel' is most effective in category Class B. The inhibitory effect of the commonly used antibiotic ceftazidime (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-negative bacteria

Microorganism	Inhibition Zone Diameter (mm) [a]								
	control $ddH_2 0$	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (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 luarate.									
Gram-Negative Bacteria									
Citrobacter koseri	NI *	NI	–	–	–	–	–	–	19.00 ± 0.18
Enterobacter cloacae	NI	NI	18.00 ± 0.57	11.00 ± 0.33	–	–	–	–	19.00 ± 0.15
Escherichia coli	NI	NI	15.67 ± 0.19	9.66 ± 0.33	4.67 ± 1.34	–	–	–	15.00 ± 0.15
E. coli ESBL	NI	NI	19.31 ± 0.19	13.00 ± 0.58	10.33 ± 0.19	9.00 ± 0.15	7.67 ± 0.19	–	0.00 ± 0.00
Klebsiella pneumoniae	NI	NI	17.00 ± 0.32	13.00 ± 0.33	9.00 ± 0.57	–	–	–	25.00 ± 0.25
Proteus vulgaris	NI	NI	–	–	–	–	–	–	25.00 ± 0.25
Pseudomonas aeruginosa	NI	NI	22.31 ± 2.50	15.32 ± 1.38	9.33 ± 0.19	9.00 ± 0.33	–	–	14.00 ± 0.12
Salmonella typhimurium	NI	NI	8.67 ± 2.83	9.33 ± 0.19	4.66 ± 1.35	–	–	–	18.00 ± 0.18
Shigella sonnei	NI	NI	12.00 ± 0.33	10.32 ± 0.18	8.67 ± 0.19	3.00 ± 1.73	3.00 ± 1.72	–	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-negative bacteria

Microorganism	Inhibition Zone Diameter (mm) [a]								
	control ddH_20	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (30 µg)
class B – Toilet Bowl Cleaners/Bleaches/Sanitizers [§] **Mr. Muscle Toilet Cleaner Duck** **Active ingredients –** Aqua, Tetrasodium EDTA, Butoxydiglycol, C9-11 pareth-6, Benzalkonium chloride, Sodium hydroxide, Parfum, Trisodium NTA, 3-(Trimethoxysiyl)-propyldimethyloctadecyl ammonium chloride, Benzyl salicylate, Alcohol, (3-Chloropropyl) trimethoxysilane, Methyl alcohol, Linalool, Lactic acid (2.02 g/100 g), Limonene, and Dimethyl stearamine.									
Gram-Negative Bacteria									
Citrobacter koseri	NI *	NI	–	–	–	–	–	–	19.00± 0.18
Enterobacter cloacae	NI	NI	–	–	–	–	–	–	19.00± 0.15
Escherichia coli	NI	NI	–	–	–	–	–	–	15.00± 0.15
E. coli ESBL	NI	NI	–	–	–	–	–	–	0.00± 0.00
Klebsiella pneumoniae	NI	NI	2.67± 1.53	–	–	–	–	–	25.00± 0.25
Proteus vulgaris	NI	NI	2.67± 1.53	–	–	–	–	–	25.00± 0.25
Pseudomonas aeruginosa	NI	NI	–	–	–	–	–	–	14.00± 0.12
Salmonella typhimurium	NI	NI	–	–	–	–	–	–	18.00± 0.18
Shigella sonnei	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 8. The inhibition zone diameter methodological analysis of the effect of toilet bowl cleaners/bleaches/sanitizers (class B) on the growth of gram-negative bacteria

Microorganism	Inhibition Zone Diameter (mm) [a]								
	control ddH_20	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (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-Negative Bacteria									
Citrobacter koseri	NI *	NI	12.00 ± 0.67	4.33 ± 1.26	–	–	–	–	19.00 ± 0.18
Enterobacter cloacae	NI	NI	11.67 ± 0.18	9.65 ± 0.19	5.66 ± 1.64	–	–	–	19.00 ± 0.15
Escherichia coli	NI	NI	10.00 ± 0.33	–	–	–	–	–	15.00 ± 0.15
E. coli ESBL	NI	NI	6.00 ± 1.73	–	–	–	–	–	0.00 ± 0.00
Klebsiella pneumoniae	NI	NI	11.67 ± 0.18	9.00 ± 0.33	8.33 ± 0.19	–	–	–	25.00 ± 0.25
Proteus vulgaris	NI	NI	–	–	–	–	–	–	25.00 ± 0.25
Pseudomonas aeruginosa	NI	NI	14.33 ± 1.34	12.00 ± 1.45	7.67 ± 0.19	5.67 ± 1.71	2.67 ± 1.54	–	14.00 ± 0.12
Salmonella typhimurium	NI	NI	10.67 ± 0.19	5.66 ± 1.71	–	–	–	–	18.00 ± 0.18
Shigella sonnei	NI	NI	9.67 ± 0.19	2.65 ± 1.51	2.67 ± 1.54	–	–	–	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.

3.2.3. The Zones of Inhibition of Class C

The effect of surface and floor mopping cleaners/detergents (category Class C) on the microbial growth of *Enterobacter cloacae* is given in Table 15 – Table 19. It is noted that 'Dettol Antiseptic/Disinfectant' is most effective in category Class C. The inhibitory effect of the commonly used antibiotic ceftazidime (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 *Enterobacter cloacae* is given in Table 20 – Table 22. It is noted that 'HiGeen Hand and Body Wash Gel' is most effective in category Class D. The inhibitory effect of the commonly used antibiotic ceftazidime (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-Negative Bacterium *Escherichia coli*

3.3.1. The Zones of Inhibition of Class A

The effect of daily mouthwash (category Class A) on the microbial growth of *Escherichia coli* is given in Table 1 – Table 5. It is noted that 'Colgate Plax Mouthwash' is most effective in category Class A. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

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

Microorganism	Inhibition Zone Diameter (mm) [a]								
	control ddH_20	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (30 µg)
class B – Toilet Bowl Cleaners/Bleaches/Sanitizers [§] **Carrefour Nettoyant Disinfectant** **Active ingredients –** Hypochlorite, Non-ionic surfactant (< 5%), and Chlorine benzalkonium.									
Gram-Negative Bacteria									
Citrobacter koseri	NI *	NI	9.00 ± 0.33	5.33 ± 1.57	–	–	–	–	19.00 ± 0.18
Enterobacter cloacae	NI	NI	12.32 ± 0.83	6.00 ± 1.76	–	–	–	–	19.00 ± 0.15
Escherichia coli	NI	NI	9.00 ± 0.57	5.00 ± 1.45	2.33 ± 1.34	–	–	–	15.00 ± 0.15
E. coli ESBL	NI	NI	2.33 ± 1.32	–	–	–	–	–	0.00 ± 0.00
Klebsiella pneumoniae	NI	NI	11.67 ± 0.38	9.67 ± 0.37	5.33 ± 1.53	4.67 ± 1.34	–	–	25.00 ± 0.25
Proteus vulgaris	NI	NI	2.67 ± 1.53	–	–	–	–	–	25.00 ± 0.25
Pseudomonas aeruginosa	NI	NI	3.00 ± 1.73	–	–	–	–	–	14.00 ± 0.12
Salmonella typhimurium	NI	NI	–	–	–	–	–	–	18.00 ± 0.18
Shigella sonnei	NI	NI	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.

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

Microorganism	**Inhibition Zone Diameter (mm) [a]**								
	control ddH_20	**control Pure Methanol**	**undiluted Disinfectant Antiseptic**	**DF 1/2**	**DF 1/4**	**DF 1/8**	**DF 1/16**	**DF 1/32**	**Ceftazidime (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-Negative Bacteria									
Citrobacter koseri	NI *	NI	–	–	–	–	–	–	19.00 ± 0.18
Enterobacter cloacae	NI	NI	–	–	–	–	–	–	19.00 ± 0.15
Escherichia coli	NI	NI	–	–	–	–	–	–	15.00 ± 0.15
E. coli ESBL	NI	NI	–	–	–	–	–	–	0.00 ± 0.00
Klebsiella pneumoniae	NI	NI	–	–	–	–	–	–	25.00 ± 0.25
Proteus vulgaris	NI	NI	–	–	–	–	–	–	25.00 ± 0.25
Pseudomonas aeruginosa	NI	NI	–	–	–	–	–	–	14.00 ± 0.12
Salmonella typhimurium	NI	NI	–	–	–	–	–	–	18.00 ± 0.18
Shigella sonnei	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.

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 *Escherichia coli* is given in Table 6 – Table 14. It is noted that 'WC Net Bleach Gel' is most effective in category Class B. The inhibitory effect of the commonly used antibiotic ceftazidime (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 *Escherichia coli* is given in Table 15 – Table 19. It is noted that 'Vim Cream Multipurpose Fast Rinsing' is most effective in category Class C. The inhibitory effect of the commonly used antibiotic ceftazidime (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 *Escherichia coli* is given in Table 20 – Table 22. It is noted that 'HiGeen Hand and

Body Wash Gel' is most effective in category Class D. The inhibitory effect of the commonly used antibiotic ceftazidime (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-Negative Bacterium *Escherichia coli* ESBL

3.4.1. The Zones of Inhibition of Class A

The effect of daily mouthwash (category Class A) on the microbial growth of *Escherichia coli* ESBL is given in Table 1 – Table 5. It is noted that 'Colgate Plax Mouthwash' is most effective in category Class A. The inhibitory effect of the commonly used antibiotic ceftazidime (30 μg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

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

Microorganism	Inhibition Zone Diameter (mm) [a]								
	control ddH$_2$0	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (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-Negative Bacteria									
Citrobacter koseri	NI *	NI	2.33 ± 1.35	–	–	–	–	–	19.00 ± 0.18
Enterobacter cloacae	NI	NI	17.00 ± 0.58	11.33 ± 0.20	9.00 ± 0.15	3.00 ± 1.73	–	–	19.00 ± 0.15
Escherichia coli	NI	NI	14.33 ± 1.02	7.67 ± 0.19	4.67 ± 1.35	–	–	–	15.00 ± 0.15
E. coli ESBL	NI	NI	9.67 ± 0.19	5.33 ± 1.54	2.33 ± 1.33	–	–	–	0.00 ± 0.00
Klebsiella pneumoniae	NI	NI	15.33 ± 0.51	9.68 ± 0.20	8.67 ± 0.19	5.32 ± 1.54	2.67 ± 1.53	–	25.00 ± 0.25
Proteus vulgaris	NI	NI	–	–	–	–	–	–	25.00 ± 0.25
Pseudomonas aeruginosa	NI	NI	14.00 ± 0.33	9.00 ± 0.32	4.67 ± 1.34	4.67 ± 1.35	–	–	14.00 ± 0.12
Salmonella typhimurium	NI	NI	13.33 ± 0.38	3.34 ± 1.92	–	–	–	–	18.00 ± 0.18
Shigella sonnei	NI	NI	9.67 ± 0.19	5.00 ± 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 12. The inhibition zone diameter methodological analysis of the effect of toilet bowl cleaners/bleaches/sanitizers (class B) on the growth of gram-negative bacteria

Microorganism	Inhibition Zone Diameter (mm) [a]								
	control ddH$_2$0	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (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-Negative Bacteria									
Citrobacter koseri	NI *	NI	14.68 ± 0.38	11.00 ± 0.05	8.00 ± 0.33	–	–	–	19.00 ± 0.18
Enterobacter cloacae	NI	NI	15.32 ± 1.01	11.67 ± 0.51	2.67 ± 1.53	–	–	–	19.00 ± 0.15
Escherichia coli	NI	NI	14.67 ± 0.96	10.00 ± 0.67	8.33 ± 0.19	–	–	–	15.00 ± 0.15
E. coli ESBL	NI	NI	9.00 ± 0.08	2.67 ± 1.54	–	–	–	–	0.00 ± 0.00
Klebsiella pneumoniae	NI	NI	17.00 ± 0.05	10.33 ± 0.19	7.33 ± 0.19	2.33 ± 1.34	–	–	25.00 ± 0.25
Proteus vulgaris	NI	NI	–	–	–	–	–	–	25.00 ± 0.25
Pseudomonas aeruginosa	NI	NI	11.67 ± 0.19	9.00 ± 0.05	3.33 ± 1.92	–	–	–	14.00 ± 0.12
Salmonella typhimurium	NI	NI	13.00 ± 0.33	9.33 ± 0.19	4.67 ± 1.34	–	–	–	18.00 ± 0.18
Shigella sonnei	NI	NI	10.33 ± 0.19	8.67 ± 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.

Table 13. The inhibition zone diameter methodological analysis of the effect of toilet bowl cleaners/bleaches/sanitizers (class B) on the growth of gram-negative bacteria

Microorganism	*Inhibition Zone Diameter (mm)* [a]								
	control ddH_20	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (30 µg)
class B – Toilet Bowl Cleaners/Bleaches/Sanitizers [§] **Spartan Max WC Lavender** **Active ingredients** – Anionic surfactant, Amphoteric surfactant, Antioxidant surfactant, Opacifier, Lanolin, Preservative, Anti-bacteria reagent, Hydrogen chloride, Fragrance, and Color.									
Gram-Negative Bacteria									
Citrobacter koseri	*NI **	*NI*	14.33 ± 0.19	11.00 ± 0.33	9.33 ± 0.50	5.00 ± 1.42	2.00 ± 1.15	–	19.00 ± 0.18
Enterobacter cloacae	*NI*	*NI*	15.67 ± 0.69	6.00 ± 1.76	–	–	–	–	19.00 ± 0.15
Escherichia coli	*NI*	*NI*	14.67 ± 0.19	9.00 ± 0.33	–	–	–	–	15.00 ± 0.15
E. coli ESBL	*NI*	*NI*	12.33 ± 0.19	2.67 ± 1.54	–	–	–	–	0.00 ± 0.00
Klebsiella pneumoniae	*NI*	*NI*	15.33 ± 1.26	5.67 ± 1.64	2.33 ± 1.34	–	–	–	25.00 ± 0.25
Proteus vulgaris	*NI*	*NI*	13.67 ± 0.51	–	–	–	–	–	25.00 ± 0.25
Pseudomonas aeruginosa	*NI*	*NI*	13.00 ± 0.08	4.00 ± 2.31	2.67 ± 1.54	–	–	–	14.00 ± 0.12
Salmonella typhimurium	*NI*	*NI*	13.00 ± 0.05	–	–	–	–	–	18.00 ± 0.18
Shigella sonnei	*NI*	*NI*	8.32 ± 0.18	–	–	–	–	–	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 14. The inhibition zone diameter methodological analysis of the effect of toilet bowl cleaners/bleaches/sanitizers (class B) on the growth of gram-negative bacteria

Microorganism	*Inhibition Zone Diameter (mm)* [a]								
	control ddH_20	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (30 µg)
class B – Toilet Bowl Cleaners/Bleaches/Sanitizers [§] **Smac Detergent Disinfectant** **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 Negative Bacteria									
Citrobacter koseri	*NI **	*NI*	5.00 ± 1.45	2.00 ± 1.15	–	–	–	–	19.00 ± 0.18
Enterobacter cloacae	*NI*	*NI*	4.67 ± 1.35	–	–	–	–	–	19.00 ± 0.15
Escherichia coli	*NI*	*NI*	10.33 ± 0.19	8.00 ± 0.05	7.00 ± 0.08	–	–	–	15.00 ± 0.15
E. coli ESBL	*NI*	*NI*	9.33 ± 0.50	5.00 ± 1.45	–	–	–	–	0.00 ± 0.00
Klebsiella pneumoniae	*NI*	*NI*	9.67 ± 0.52	9.00 ± 0.58	8.00 ± 0.33	2.33 ± 1.34	–	–	25.00 ± 0.25
Proteus vulgaris	*NI*	*NI*	–	–	–	–	–	–	25.00 ± 0.25
Pseudomonas aeruginosa	*NI*	*NI*	–	–	–	–	–	–	14.00 ± 0.12
Salmonella typhimurium	*NI*	*NI*	–	–	–	–	–	–	18.00 ± 0.18
Shigella sonnei	*NI*	*NI*	9.67 ± 0.38	9.33 ± 0.37	8.32 ± 0.18	–	–	–	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.

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 *Escherichia coli* ESBL is given in Table 6 – Table 14. It is noted that 'WC Net Bleach Gel' is most effective in category Class B. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

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 *Escherichia coli* ESBL is given in Table 15 – Table 19. It is noted that 'Dettol Antiseptic/Disinfectant' is most effective in category Class C. The inhibitory effect of the commonly used antibiotic ceftazidime (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-negative bacteria

Microorganism	Inhibition Zone Diameter (mm) [a]								
	control ddH_20	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (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-Negative Bacteria									
Citrobacter koseri	NI *	NI	7.00 ± 0.33	2.00 ± 1.15	2.00 ± 0.05	2.00 ± 1.15	2.00 ± 1.15	–	19.00 ± 0.18
Enterobacter cloacae	NI	NI	14.33 ± 0.70	10.00 ± 0.05	5.33 ± 1.54	–	–	–	19.00 ± 0.15
Escherichia coli	NI	NI	10.67 ± 0.51	8.00 ± 0.05	–	–	–	–	15.00 ± 0.15
E. coli ESBL	NI	NI	9.33 ± 0.19	5.67 ± 1.64	–	–	–	–	0.00 ± 0.00
Klebsiella pneumoniae	NI	NI	16.67 ± 0.77	10.00 ± 2.96	11.00 ± 1.20	7.00 ± 2.03	–	–	25.00 ± 0.25
Proteus vulgaris	NI	NI	11.67 ± 0.50	5.66 ± 3.27	3.00 ± 1.73	–	–	–	25.00 ± 0.25
Pseudomonas aeruginosa	NI	NI	2.15 ± 1.35	–	–	–	–	–	14.00 ± 0.12
Salmonella typhimurium	NI	NI	10.00 ± 0.05	–	–	–	–	–	18.00 ± 0.18
Shigella sonnei	NI	NI	10.00 ± 0.33	8.67 ± 0.19	8.00 ± 0.05	2.33 ± 1.34	2.32 ± 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 16. The inhibition zone diameter methodological analysis of the effect of surface and floor mopping cleaners/detergents (class C) on the growth of gram-negative bacteria

Microorganism	Inhibition Zone Diameter (mm) [a]								
	control ddH_20	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (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-Negative Bacteria									
Citrobacter koseri	NI *	NI	2.33± 1.35	–	–	–	–	–	19.00± 0.18
Enterobacter cloacae	NI	NI	10.00± 0.33	2.67± 1.54	–	–	–	–	19.00± 0.15
Escherichia coli	NI	NI	9.33± 0.83	–	–	–	–	–	15.00± 0.15
E. coli ESBL	NI	NI	–	–	–	–	–	–	0.00± 0.00
Klebsiella pneumoniae	NI	NI	6.67± 1.95	2.67± 1.54	–	–	–	–	25.00± 0.25
Proteus vulgaris	NI	NI	3.00± 1.50	3.00± 1.73	3.32± 1.92	–	–	–	25.00± 0.25
Pseudomonas aeruginosa	NI	NI	–	–	–	–	–	–	14.00± 0.12
Salmonella typhimurium	NI	NI	–	–	–	–	–	–	18.00± 0.18
Shigella sonnei	NI	NI	2.67± 1.54	–	–	–	–	–	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.

3.4.4. The Zones of Inhibition of Class D

The effect of hand and body wash gels (category Class D) on the microbial growth of *Escherichia coli* ESBL is given in Table 20 – Table 22. It is noted that 'HiGeen Hand and Body Wash Gel' is most effective in category Class D. The inhibitory effect of the commonly used antibiotic ceftazidime (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-Negative Bacterium *Klebsiella pneumoniae*

3.5.1. The Zones of Inhibition of Class A

The effect of daily mouthwash (category Class A) on the microbial growth of *Klebsiella pneumoniae* is given in Table 1 – Table 5. It is noted that 'Colgate Plax Mouthwash' is most effective in category Class A. The inhibitory effect of the commonly used antibiotic ceftazidime (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 *Klebsiella pneumoniae* is given in Table 6 – Table 14. It is noted that 'WC Net Bleach Gel' is comparably as effective as 'Harpic Power Plus Disinfectant' in category Class B. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

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-negative bacteria

Microorganism	Inhibition Zone Diameter (mm) [a]								
	control ddH_20	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (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 pareth-6, Butoxydiglycol, C11-C13 isoparaffin, Sodium cocoate, Parfum, Carbomer, Benzisothiazolinone, and CI 47005.									
Gram-Negative Bacteria									
Citrobacter koseri	NI *	NI	–	–	–	–	–	–	19.00± 0.18
Enterobacter cloacae	NI	NI	8.33± 2.54	–	–	–	–	–	19.00± 0.15
Escherichia coli	NI	NI	14.67± 0.19	9.00± 0.33	–	–	–	–	15.00± 0.15
E. coli ESBL	NI	NI	–	–	–	–	–	–	0.00± 0.00
Klebsiella pneumoniae	NI	NI	–	–	–	–	–	–	25.00± 0.25
Proteus vulgaris	NI	NI	–	–	–	–	–	–	25.00± 0.25
Pseudomonas aeruginosa	NI	NI	–	–	–	–	–	–	14.00± 0.12
Salmonella typhimurium	NI	NI	5.33± 1.57	–	–	–	–	–	18.00± 0.18
Shigella sonnei	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 18. The inhibition zone diameter methodological analysis of the effect of surface and floor mopping cleaners/detergents (class C) on the growth of gram-negative bacteria

Microorganism	Inhibition Zone Diameter (mm) [a]								
	control ddH_20	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (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-Negative Bacteria									
Citrobacter koseri	NI *	NI	2.67± 1.53	2.00± 1.15	–	–	–	–	19.00± 0.18
Enterobacter cloacae	NI	NI	–	–	–	–	–	–	19.00± 0.15
Escherichia coli	NI	NI	7.68± 0.21	–	–	–	–	–	15.00± 0.15
E. coli ESBL	NI	NI	–	–	–	–	–	–	0.00± 0.00
Klebsiella pneumoniae	NI	NI	–	–	–	–	–	–	25.00± 0.25
Proteus vulgaris	NI	NI	5.33± 1.53	–	–	–	–	–	25.00± 0.25
Pseudomonas aeruginosa	NI	NI	–	–	–	–	–	–	14.00± 0.12
Salmonella typhimurium	NI	NI	–	–	–	–	–	–	18.00± 0.18
Shigella sonnei	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.

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 *Klebsiella pneumoniae* is given in Table 15 – Table 19. It is noted that 'Dettol Antiseptic/Disinfectant' is most effective in category Class C. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

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 *Klebsiella pneumoniae* is given in Table 20 – Table 22. It is noted that 'HiGeen Hand and Body Wash Gel' is most effective in category Class D. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.6. The Zones of Inhibition of Gram-Negative Bacterium *Proteus vulgaris*

3.6.1. The Zones of Inhibition of Class A

The effect of daily mouthwash (category Class A) on the microbial growth of *Proteus vulgaris* is given in Table 1 – Table 5. It is noted that 'Colgate Plax Mouthwash' is most effective in category Class A. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.6.2. The Zones of Inhibition of Class B

The effect of toilet bowl cleaners/bleaches/sanitizers (category Class B) on the microbial growth of *Proteus vulgaris* is given in Table 6 – Table 14. It is noted that 'Spartan Max WC Lavender' is most effective in category Class B. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.6.3. The Zones of Inhibition of Class C

The effect of surface and floor mopping cleaners/detergents (category Class C) on the microbial growth of *Proteus vulgaris* is given in Table 15 – Table 19. It is noted that 'Dettol Antiseptic/Disinfectant' is most effective in category Class C. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.6.4. The Zones of Inhibition of Class D

The effect of hand and body wash gels (category Class D) on the microbial growth of *Proteus vulgaris* is given in Table 20 – Table 22. It is noted that 'HiGeen Hand and Body Wash Gel' is most effective in category Class D. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.7. The Zones of Inhibition of Gram-Negative Bacterium *Pseudomonas aeruginosa*

3.7.1. The Zones of Inhibition of Class A

The effect of daily mouthwash (category Class A) on the microbial growth of *Pseudomonas aeruginosa* is given in Table 1 – Table 5. It is noted that 'Perio.Kin Chlorhexidina 0.20%' is most effective in category Class A. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.7.2. The Zones of Inhibition of Class B

The effect of toilet bowl cleaners/bleaches/sanitizers (category Class B) on the microbial growth of *Pseudomonas aeruginosa* is given in Table 6 – Table 14. It is noted that 'WC Net Bleach Gel' is most effective in category Class B. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

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-negative bacteria

Microorganism	Inhibition Zone Diameter (mm) [a]								
	control ddH_20	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (30 µg)
class C – Surface and Floor Mopping Cleaners/Detergents [§] Ajax Fete des Fleurs **Active ingredients** – Anionic surfactants (< 5%), Non-ionic surfactants, Perfume, Buthylphenyl methylpropional, Hexyl cinnamal, Citronellol, Linalool, Geraniol, Glutaral, Methylchloroisothiazolinone, Methylisothiazolinone, and Octylisothiazolinone.									
Gram-Negative Bacteria									
Citrobacter koseri	NI *	NI	–	–	–	–	–	–	19.00 ± 0.18
Enterobacter cloacae	NI	NI	–	–	–	–	–	–	19.00 ± 0.15
Escherichia coli	NI	NI	–	–	–	–	–	–	15.00 ± 0.15
E. coli ESBL	NI	NI	–	–	–	–	–	–	0.00 ± 0.00
Klebsiella pneumoniae	NI	NI	–	–	–	–	–	–	25.00 ± 0.25
Proteus vulgaris	NI	NI	–	–	–	–	–	–	25.00 ± 0.25
Pseudomonas aeruginosa	NI	NI	–	–	–	–	–	–	14.00 ± 0.12
Salmonella typhimurium	NI	NI	–	–	–	–	–	–	18.00 ± 0.18
Shigella sonnei	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.

3.7.3. The Zones of Inhibition of Class C

The effect of surface and floor mopping cleaners/detergents (category Class C) on the microbial growth of *Pseudomonas aeruginosa* is given in Table 15 – Table 19. It is noted that 'Dettol Antiseptic/Disinfectant' is the only detergent that is minimally effective in category Class C. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.7.4. The Zones of Inhibition of Class D

The effect of hand and body wash gels (category Class D) on the microbial growth of *Pseudomonas aeruginosa* is given in Table 20 – Table 22. It is noted that 'HiGeen Hand and Body Wash Gel' is most effective in category Class D. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.8. The Zones of Inhibition of Gram-Negative Bacterium *Salmonella typhimurium*

3.8.1. The Zones of Inhibition of Class A

The effect of daily mouthwash (category Class A) on the microbial growth of *Salmonella typhimurium* is given in Table 1 – Table 5. It is noted that 'Colgate Plax Mouthwash' is most effective in category Class A. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.8.2. The Zones of Inhibition of Class B

The effect of toilet bowl cleaners/bleaches/sanitizers (category Class B) on the microbial growth of *Salmonella typhimurium* is given in Table 6 – Table 14. It is noted that 'Clorox Bleach Rain Clean' is most effective in category Class B, but as nearly as effective as 'Harpic Power Plus Disinfectant' and 'Spartan Max WC Lavender'. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.8.3. The Zones of Inhibition of Class C

The effect of surface and floor mopping cleaners/detergents (category Class C) on the microbial growth of *Salmonella typhimurium* is given in Table 15 – Table 19. It is noted that 'Dettol Antiseptic/Disinfectant' is the only detergent that is minimally effective in category Class C. The inhibitory effect of the commonly used antibiotic ceftazidime (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-negative bacteria

Microorganism	Inhibition Zone Diameter (mm) [a]								
	control ddH_20	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (30 µg)
class D – Hand and Body Wash Gels [§] Lifebuoy Hand and Body Wash Gel (Antiseptic) Active ingredients – Glycerine, and Vitamin beads.									
Gram-Negative Bacteria									
Citrobacter koseri	NI *	NI	–	–	–	–	–	–	19.00 ± 0.18
Enterobacter cloacae	NI	NI	–	–	–	–	–	–	19.00 ± 0.15
Escherichia coli	NI	NI	–	–	–	–	–	–	15.00 ± 0.15
E. coli ESBL	NI	NI	–	–	–	–	–	–	0.00 ± 0.00
Klebsiella pneumoniae	NI	NI	–	–	–	–	–	–	25.00 ± 0.25
Proteus vulgaris	NI	NI	–	–	–	–	–	–	25.00 ± 0.25
Pseudomonas aeruginosa	NI	NI	3.33 ± 1.92	–	–	–	–	–	14.00 ± 0.12
Salmonella typhimurium	NI	NI	–	–	–	–	–	–	18.00 ± 0.18
Shigella sonnei	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-negative bacteria

Microorganism	Inhibition Zone Diameter (mm) [a]								
	control ddH_20	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (30 µg)
class D – Hand and Body Wash Gels [§] HiGeen Hand and Body Wash Gel (Antiseptic) Active ingredients – Glycerine, and Vitamin beads.									
Gram-Negative Bacteria									
Citrobacter koseri	NI *	NI	44.33 ± 3.27	36.00 ± 4.04	26.67 ± 0.19	19.00 ± 0.33	18.33 ± 0.77	14.32 ± 0.69	19.00 ± 0.18
Enterobacter cloacae	NI	NI	50.00 ± 0.50	35.33 ± 4.22	33.32 ± 4.91	34.67 ± 4.42	33.68 ± 4.71	30.65 ± 5.59	19.00 ± 0.15
Escherichia coli	NI	NI	50.00 ± 0.50	42.33 ± 4.42	23.00 ± 0.88	21.67 ± 1.35	18.00 ± 0.33	18.32 ± 0.19	15.00 ± 0.15
E. coli ESBL	NI	NI	50.00 ± 0.50	42.66 ± 4.23	32.00 ± 5.20	22.00 ± 0.33	21.33 ± 0.50	16.33 ± 1.54	0.00 ± 0.00
Klebsiella pneumoniae	NI	NI	42.00 ± 4.62	34.00 ± 4.63	26.67 ± 2.04	24.33 ± 1.71	21.67 ± 0.51	29.67 ± 5.92	25.00 ± 0.25
Proteus vulgaris	NI	NI	50.00 ± 0.65	41.67 ± 4.81	40.00 ± 5.77	40.00 ± 5.71	18.67 ± 2.16	16.00 ± 2.52	25.00 ± 0.25
Pseudomonas aeruginosa	NI	NI	9.00 ± 0.32	–	–	–	–	–	14.00 ± 0.12
Salmonella typhimurium	NI	NI	50.00 ± 0.50	50.00 ± 0.55	42.00 ± 4.61	40.33 ± 5.58	41.67 ± 4.81	50.00 ± 1.58	18.00 ± 0.18
Shigella sonnei	NI	NI	26.00 ± 3.06	22.00 ± 3.71	16.67 ± 1.92	14.67 ± 2.03	9.00 ± 2.64	6.67 ± 1.92	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.

3.8.4. The Zones of Inhibition of Class D

The effect of hand and body wash gels (category Class D) on the microbial growth of *Salmonella typhimurium* is given in Table 20 – Table 22. It is noted that 'HiGeen Hand and Body Wash Gel' is most effective in category Class D. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

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

Microorganism	Inhibition Zone Diameter (mm) [a]								
	control ddH_20	control Pure Methanol	undiluted Disinfectant Antiseptic	DF 1/2	DF 1/4	DF 1/8	DF 1/16	DF 1/32	Ceftazidime (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-Negative Bacteria									
Citrobacter koseri	NI *	NI	–	–	–	–	–	–	19.00 ± 0.18
Enterobacter cloacae	NI	NI	6.67 ± 1.92	3.33 ± 1.91	–	–	–	–	19.00 ± 0.15
Escherichia coli	NI	NI	5.67 ± 1.64	–	–	–	–	–	15.00 ± 0.15
E. coli ESBL	NI	NI	2.67 ± 1.53	–	–	–	–	–	0.00 ± 0.00
Klebsiella pneumoniae	NI	NI	4.00 ± 2.31	–	–	–	–	–	25.00 ± 0.25
Proteus vulgaris	NI	NI	–	–	–	–	–	–	25.00 ± 0.25
Pseudomonas aeruginosa	NI	NI	–	–	–	–	–	–	14.00 ± 0.12
Salmonella typhimurium	NI	NI	2.33 ± 1.34	–	–	–	–	–	18.00 ± 0.18
Shigella sonnei	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.

3.9. The Zones of Inhibition of Gram-Negative Bacterium *Shigella sonnei*

3.9.1. The Zones of Inhibition of Class A

The effect of daily mouthwash (category Class A) on the microbial growth of *Shigella sonnei* is given in Table 1 – Table 5. It is noted that 'Colgate Plax Mouthwash' is most effective in category Class A. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.9.2. The Zones of Inhibition of Class B

The effect of toilet bowl cleaners/bleaches/sanitizers (category Class B) on the microbial growth of *Shigella sonnei* is given in Table 6 – Table 14. It is noted that 'WC Net Bleach Gel' is most effective in category Class B. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.9.3. The Zones of Inhibition of Class C

The effect of surface and floor mopping cleaners/detergents (category Class C) on the microbial growth of *Shigella sonnei* is given in Table 15 – Table 19. It is noted that 'Dettol Antiseptic/Disinfectant' is the only detergent that is minimally effective in category Class C. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.9.4. The Zones of Inhibition of Class D

The effect of hand and body wash gels (category Class D) on the microbial growth of *Shigella sonnei* is given in Table 20 – Table 22. It is noted that 'HiGeen Hand and Body Wash Gel' is most effective in category Class D. The inhibitory effect of the commonly used antibiotic ceftazidime (30 µg) is set as a reference for comparison as a positive control, while absolute methanol is recognized as negative control.

3.10. The Comparative Analytical Assessment of Various Household Disinfectants

Comparative analytical assessment of the zones of inhibition of various classes (A – D) with reference to ceftazidime (30 µg) depicts the efficacious impact of those antiseptics and disinfectants against pathogenic bacteria. The zones of inhibition of classes A – D for *Citrobacter koseri* is shown in Figure 1. Similarly, the zones of inhibition of classes A – D for *Enterobacter cloacae* is shown in Figure 2. The zones of inhibition of classes A – D for *Escherichia coli* is shown in Figure 3. The zones of inhibition of classes A – D for *Escherichia coli* ESBL is shown in Figure 4. The zones of inhibition of classes A – D for *Klebsiella pneumoniae* is shown in Figure 5. The zones of inhibition of classes A – D for *Proteus vulgaris* is shown in Figure 6. The zones of inhibition of classes A – D for *Pseudomonas aeruginosa* is shown in Figure 7. The zones of inhibition of classes A – D for *Salmonella typhimurium* is shown in Figure 8. The zones of inhibition of classes A – D for *Shigella sonnei* is shown in Figure 9. These results have been calculated based on the method described in [1].

3.11. 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 $E_R = Zone_{max} / Zone_{min}$. 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 10. The E_R of Class B is shown in Figure 11. The E_R of Class C is shown in Figure 12. The E_R of Class

D is shown in Figure 13. These results have been calculated based on the method described in [1].

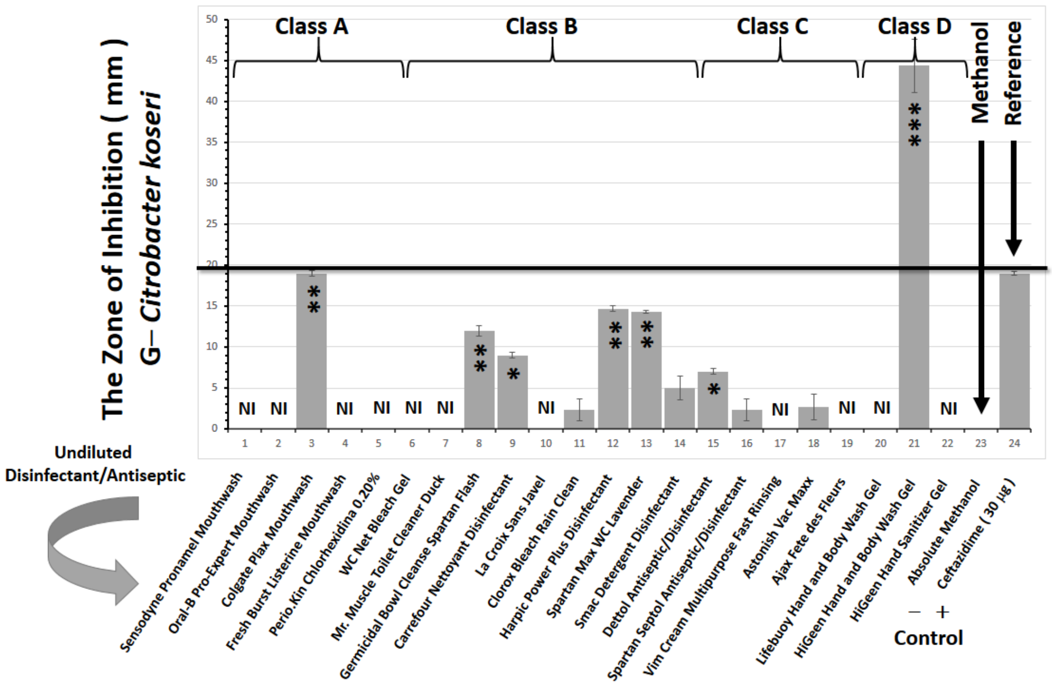

Figure 1. Depictive comparative assessment of the antimicrobial efficacy of various detergents against gram-negative *Citrobacter koseri* bacteria, as compared with ceftazidime (30 μg). The zone of inhibition of ceftazidime was set as a reference (lane 24; horizontal straight line), and that for absolute 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 ceftazidime, 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 ceftazidime or absolute MetOH. NI = No inhibition

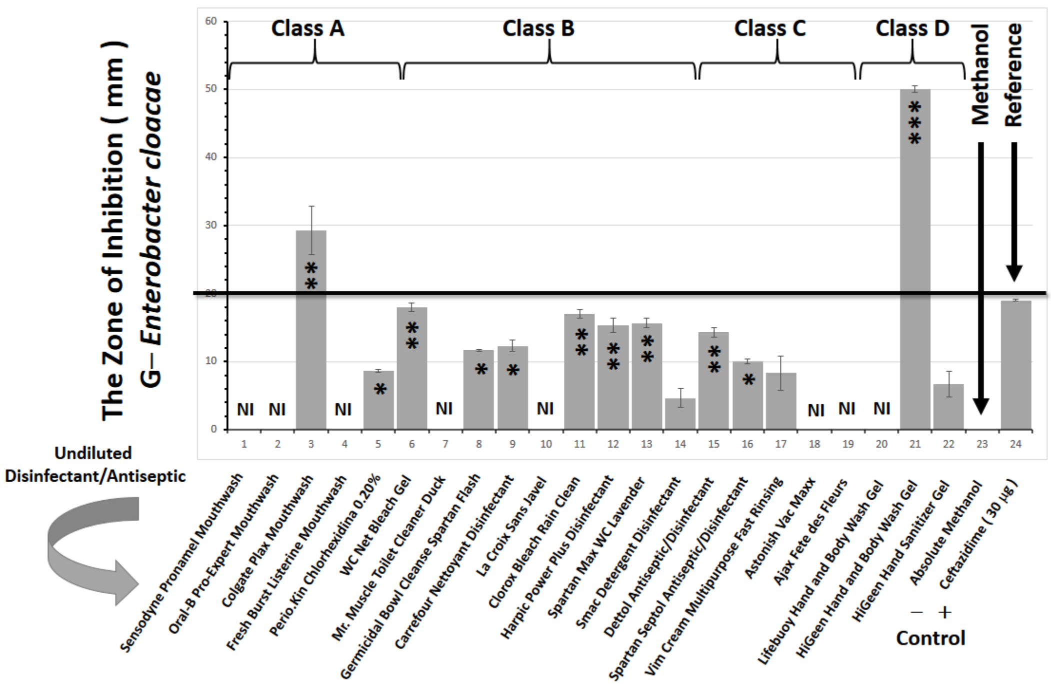

Figure 2. Depictive comparative assessment of the antimicrobial efficacy of various detergents against gram-negative *Enterobacter cloacae* bacteria, as compared with ceftazidime (30 μg). The zone of inhibition of ceftazidime was set as a reference (lane 24; horizontal straight line), and that for absolute 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 ceftazidime, 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 ceftazidime or absolute MetOH. NI = No inhibition

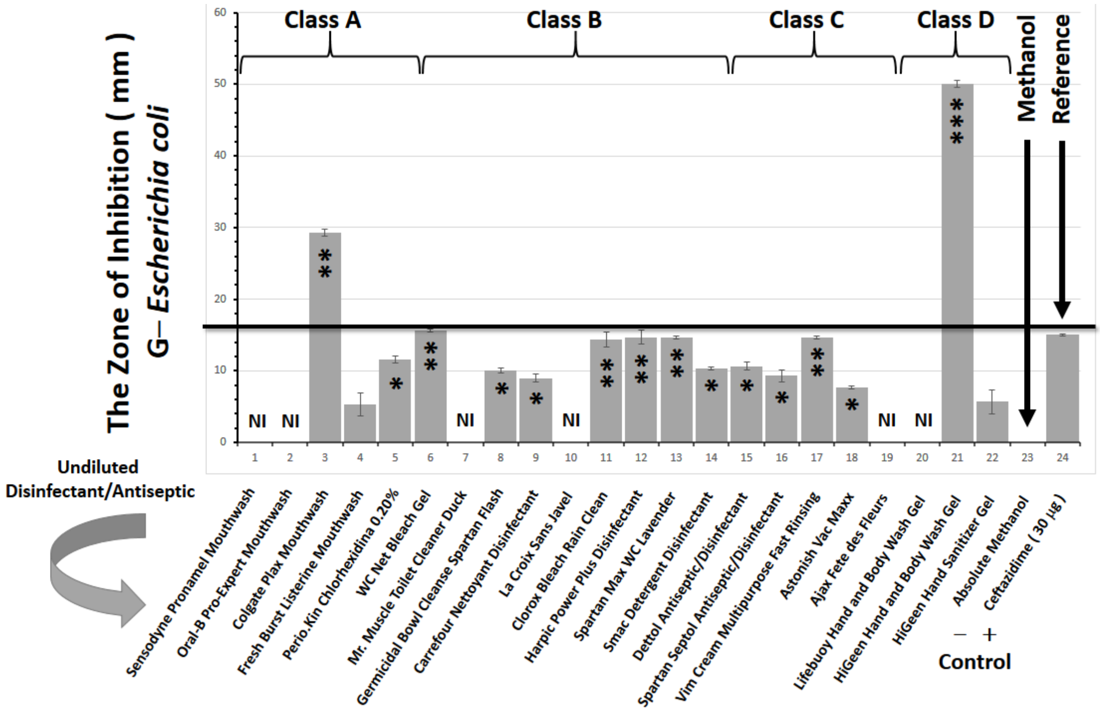

Figure 3. Depictive comparative assessment of the antimicrobial efficacy of various detergents against gram-negative *Escherichia coli* bacteria, as compared with ceftazidime (30 µg). The zone of inhibition of ceftazidime was set as a reference (lane 24; horizontal straight line), and that for absolute 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 ceftazidime, 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 ceftazidime or absolute MetOH. NI = No inhibition

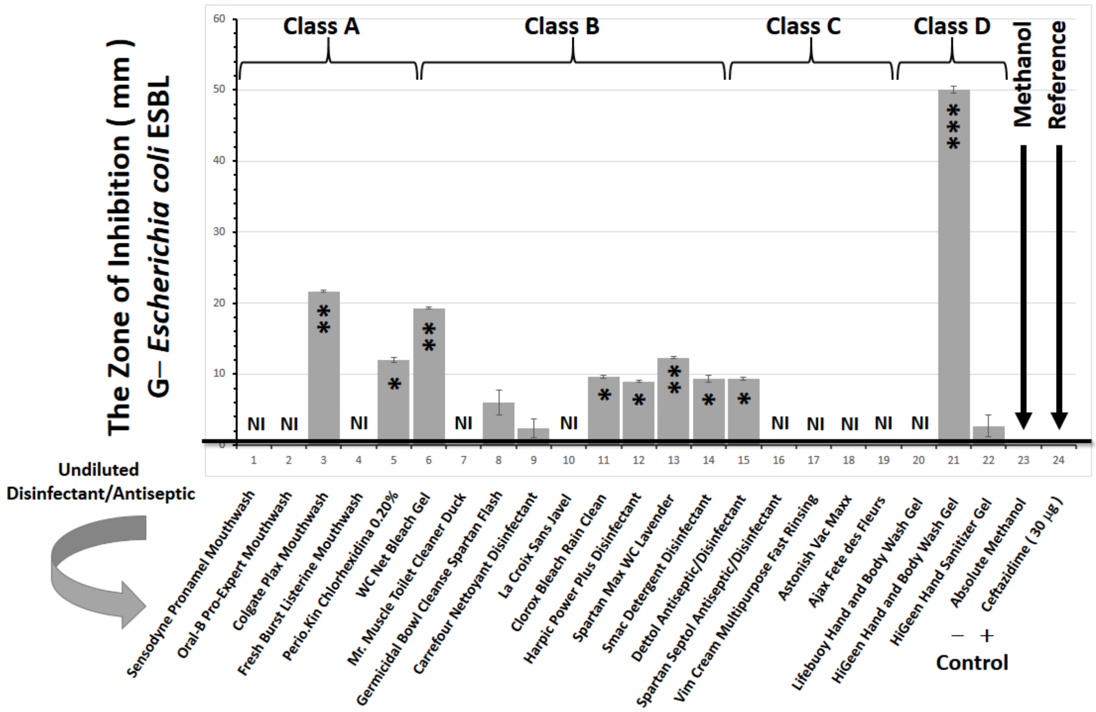

Figure 4. Depictive comparative assessment of the antimicrobial efficacy of various detergents against gram-negative *Escherichia coli* ESBL bacteria, as compared with ceftazidime (30 µg). The zone of inhibition of ceftazidime was set as a reference (lane 24; horizontal straight line), and that for absolute 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 ceftazidime, 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 ceftazidime or absolute MetOH. NI = No inhibition

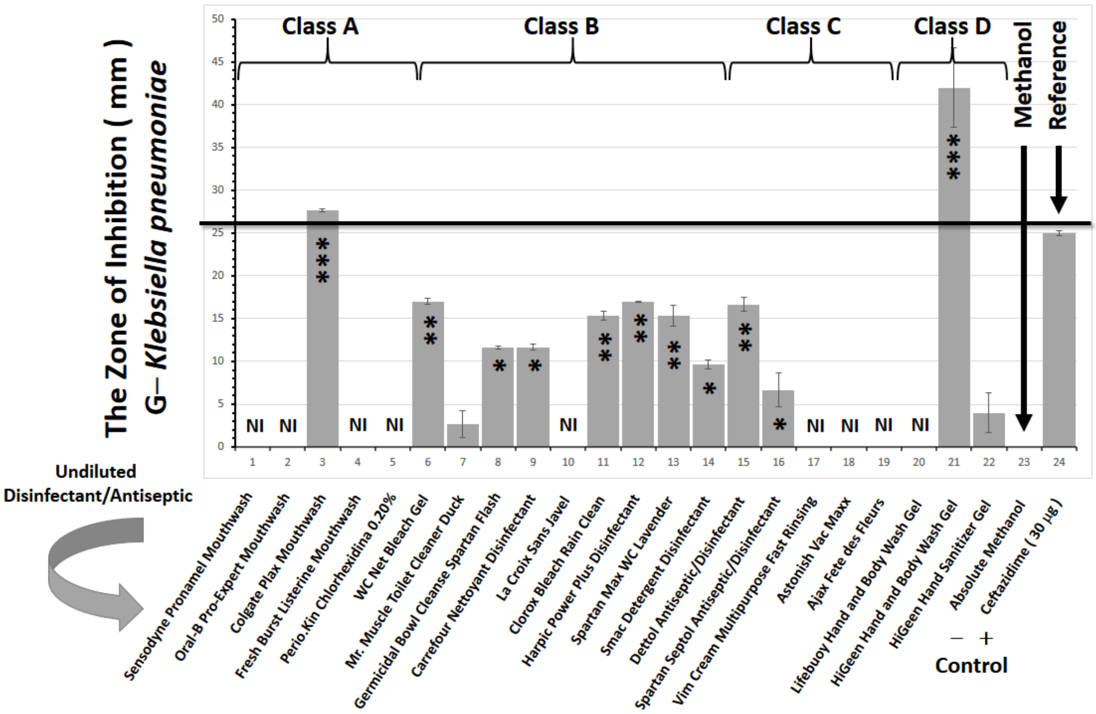

Figure 5. Depictive comparative assessment of the antimicrobial efficacy of various detergents against gram-negative *Klebsiella pneumoniae* bacteria, as compared with ceftazidime (30 µg). The zone of inhibition of ceftazidime was set as a reference (lane 24; horizontal straight line), and that for absolute 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 ceftazidime, 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 ceftazidime or absolute MetOH. NI = No inhibition

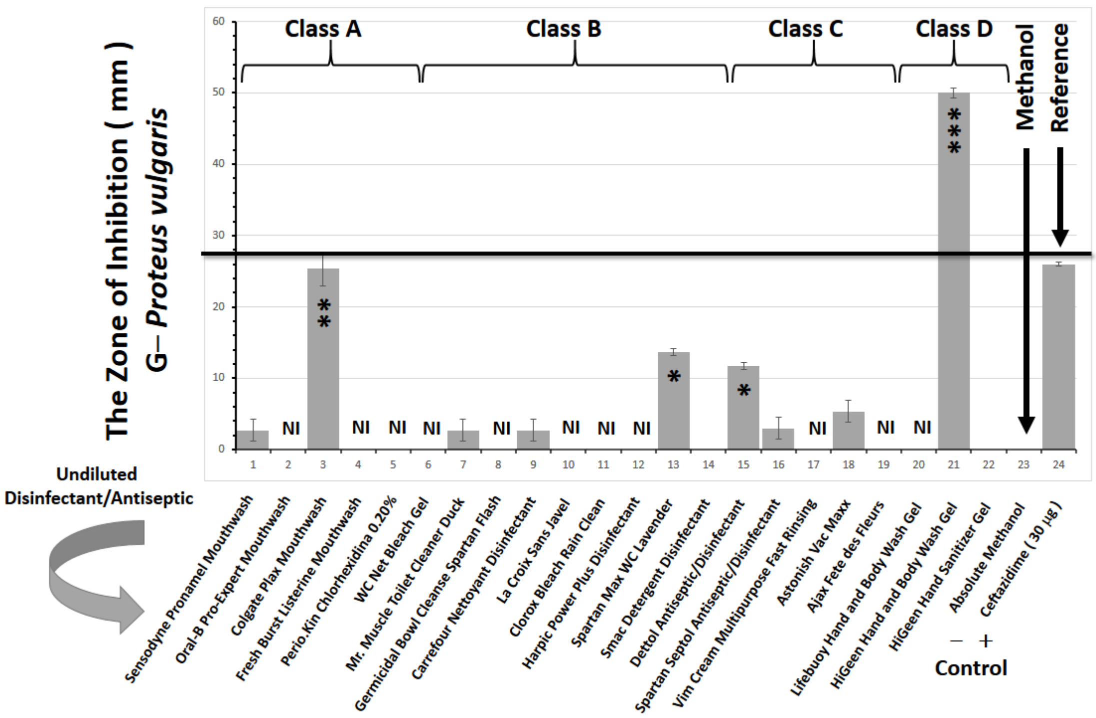

Figure 6. Depictive comparative assessment of the antimicrobial efficacy of various detergents against gram-negative *Proteus vulgaris* bacteria, as compared with ceftazidime (30 µg). The zone of inhibition of ceftazidime was set as a reference (lane 24; horizontal straight line), and that for absolute 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 ceftazidime, 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 ceftazidime or absolute MetOH. NI = No inhibition

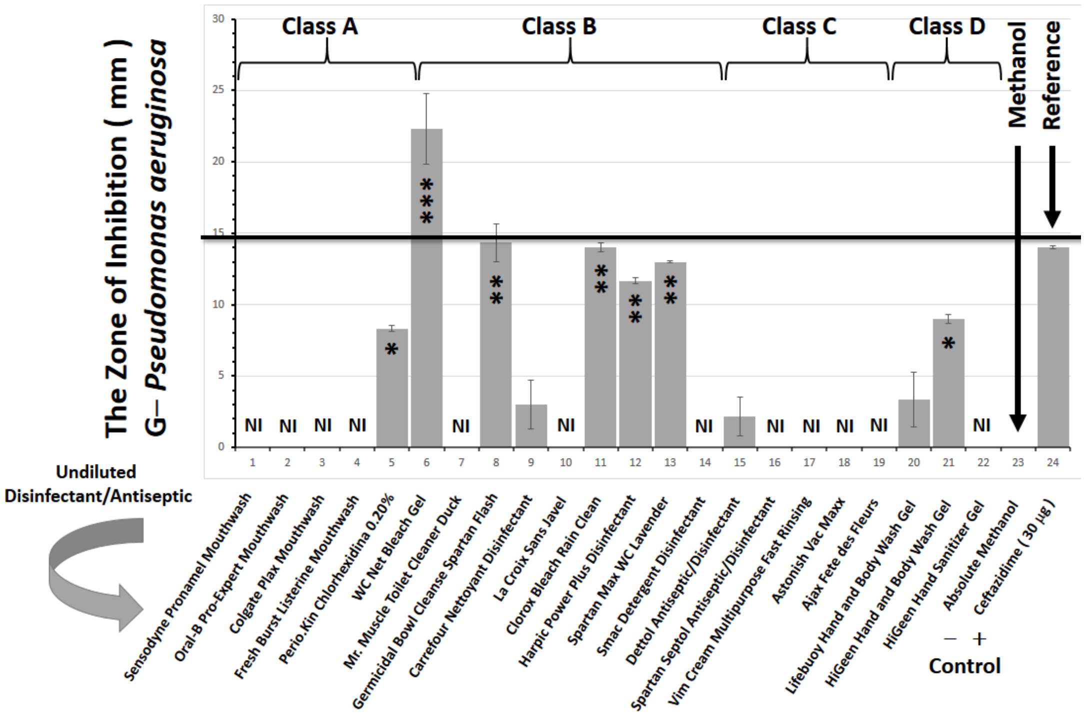

Figure 7. Depictive comparative assessment of the antimicrobial efficacy of various detergents against gram-negative *Pseudomonas aeruginosa* bacteria, as compared with ceftazidime (30 µg). The zone of inhibition of ceftazidime was set as a reference (lane 24; horizontal straight line), and that for 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 ceftazidime, 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 ceftazidime or absolute MetOH. NI = No inhibition

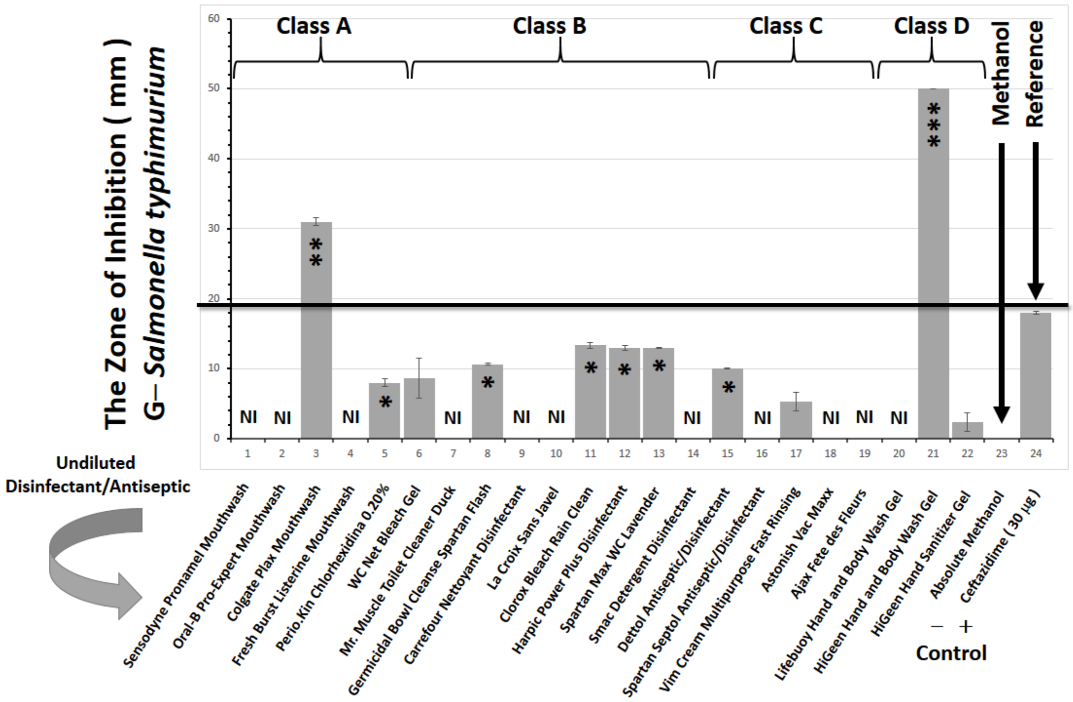

Figure 8. Depictive comparative assessment of the antimicrobial efficacy of various detergents against gram-negative *Salmonella typhimurium* bacteria, as compared with ceftazidime (30 µg). The zone of inhibition of ceftazidime was set as a reference (lane 24; horizontal straight line), and that for 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 ceftazidime, 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 ceftazidime or absolute MetOH. NI = No inhibition

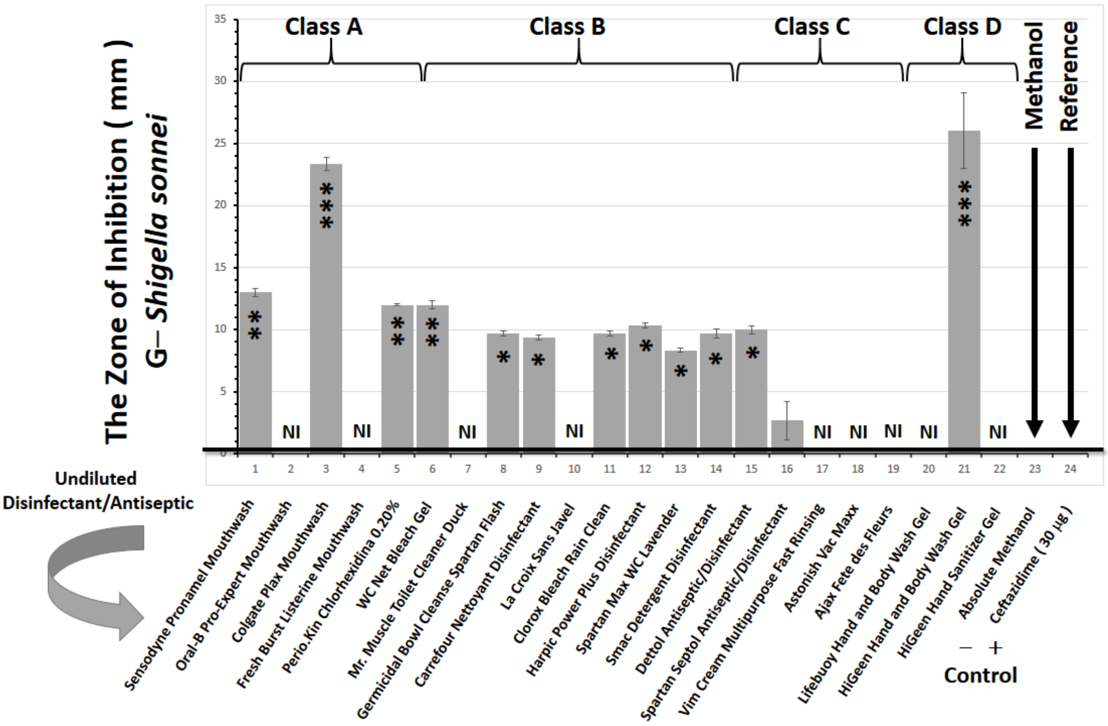

Figure 9. Depictive comparative assessment of the antimicrobial efficacy of various detergents against gram-negative *Shigella sonnei* bacteria, as compared with ceftazidime (30 μg). The zone of inhibition of ceftazidime was set as a reference (lane 24; horizontal straight line), and that for absolute 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 ceftazidime, 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 ceftazidime or absolute MetOH. NI = No inhibition

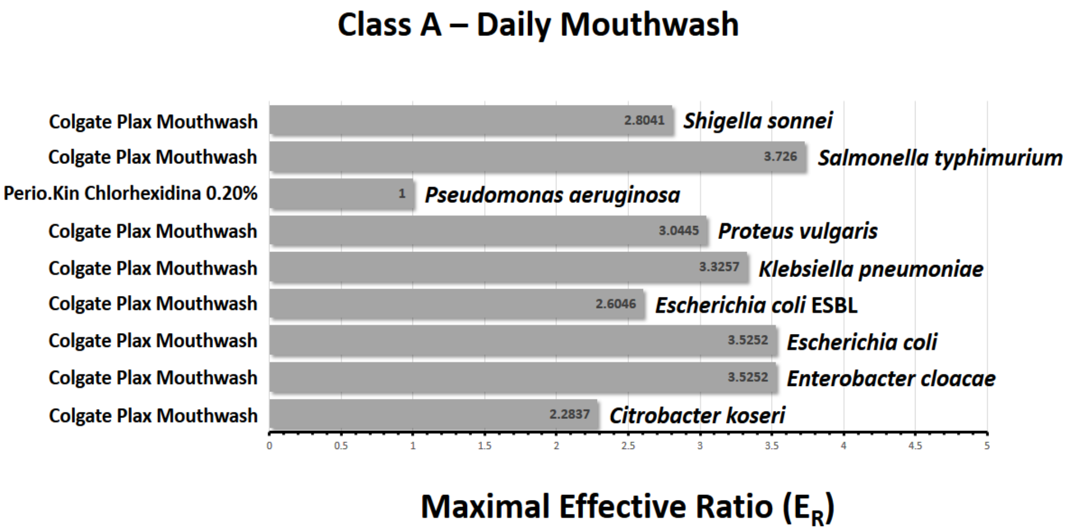

Figure 10. The putative immunomodulatory/anti-inflammatory, anti-microbial and bactericidal mechanisms are estimated by determining the probable effective ratios. The maximal effective ratio (E_R) of Class A (Daily Mouthwash) on gram-negative bacteria. E_R was calculated as the ratio of each bacterium with maximal zone of inhibition against the minimum zone of inhibition (set as 1) within the same category, such that $E_R = Zone_{max} / Zone_{min}$. 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 *E. coli* is 'Colgate Plax Mouthwash.' The number of experimental observations is n = 3

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

Typical microbial growth of gram-negative bacteria in the presence of commercially available disinfectants and antiseptics in culture is shown in Figure 14. The growth of *Citrobacter koseri* 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 14A. The growth of *Enterobacter cloacae* in the presence of 'WC Net Bleach Gel' at various concentrations (undiluted, 1/2, 1/4, 1/8, 1/16, and 1/32 + positive control, ceftazidime (30 μg)), noting zones of inhibition is shown in Figure 14B. The growth of *Escherichia coli* in the presence of 'Colgate Plax Mouthwash' at various concentrations (undiluted, 1/2, 1/4, 1/8, 1/16, and 1/32 + positive control, ceftazidime (30 μg)), noting zones of inhibition is shown in Figure 14C. The growth of *Escherichia coli* ESBL 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 14D. The growth of *Klebsiella pneumoniae* 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, ceftazidime (30 μg)), noting zones of inhibition is shown in Figure 14E. The growth of *Proteus vulgaris* in the presence of 'Spartan Max WC Lavender' 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 14F. The growth of *Pseudomonas aeruginosa* in the presence of 'WC Net Bleach 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 14G. The growth of *Salmonella typhimurium* 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 14H. The growth of *Shigella sonnei* in the presence of 'Perio.Kin Chlorhexidina' 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 14I.

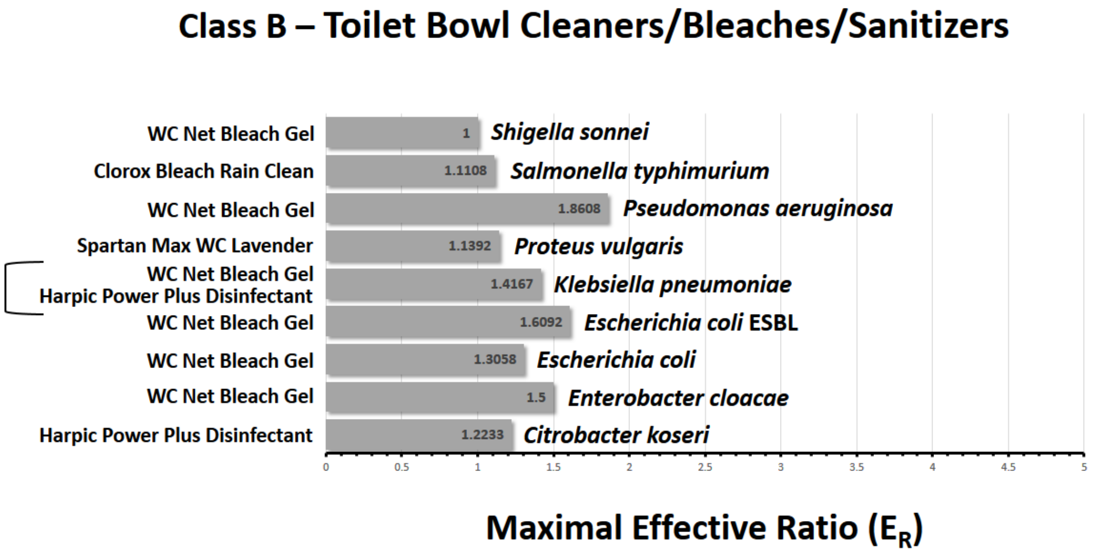

Figure 11. The putative immunomodulatory/anti-inflammatory, anti-microbial and bactericidal mechanisms are estimated by determining the probable effective ratios. The maximal effective ratio (E_R) of Class B (Toilet Bowl Cleaners/Bleaches/Sanitizers) on gram-negative bacteria. E_R was calculated as the 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 = Zone $_{max}$ / 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 Toilet Bowl Cleaners/Bleaches/Sanitizers against *E. coli* is 'WC Net Bleach Gel.' The number of experimental observations is n = 3

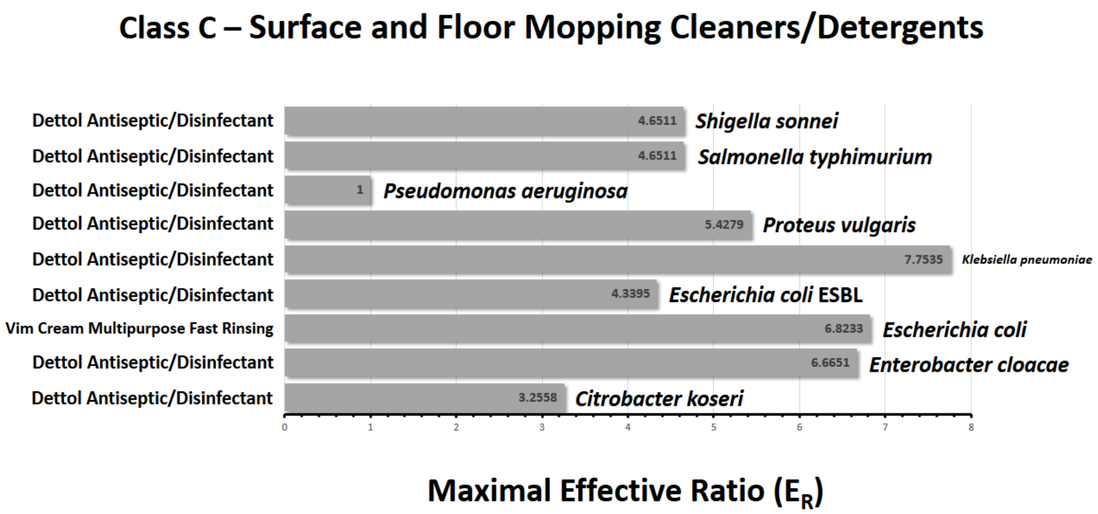

Figure 12. The putative immunomodulatory/anti-inflammatory, anti-microbial and bactericidal mechanisms are estimated by determining the probable effective ratios. The maximal effective ratio (E_R) of Class C (Surface and Floor Mopping Cleaners/Detergents) on gram-negative bacteria. E_R was calculated 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 = Zone $_{max}$ / 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 Surface and Floor Mopping Cleaners/Detergents against *E. coli* is 'Vim Cream Multipurpose Fast Rinsing.' The number of experimental observations is n = 3

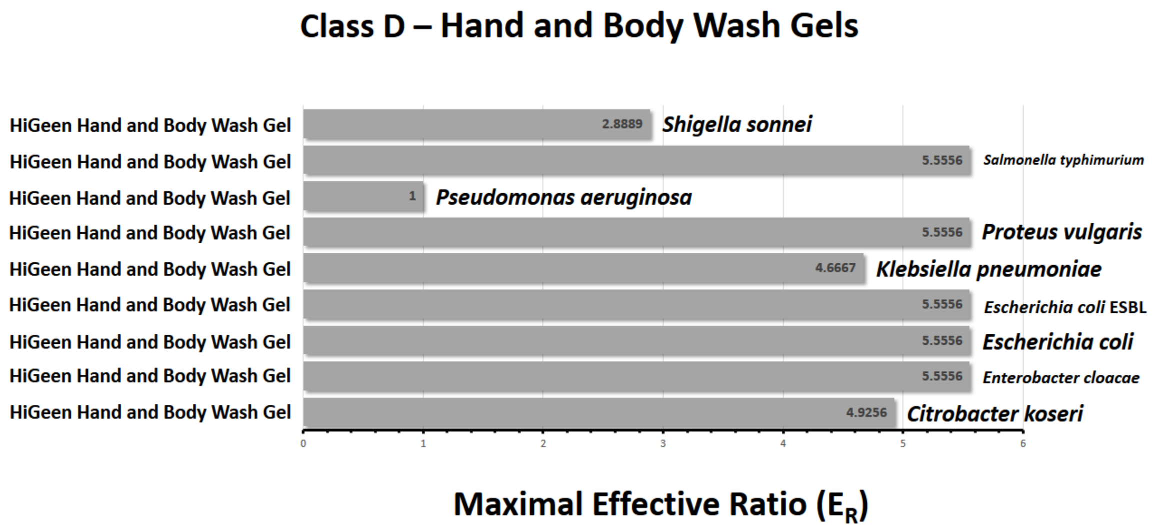

Figure 13. The putative immunomodulatory/anti-inflammatory, anti-microbial and bactericidal mechanisms are estimated by determining the probable effective ratios. The maximal effective ratio (E_R) of Class D (Hand and Body Wash Gels) on gram-negative bacteria. E_R was calculated as the ratio of each bacterium with maximal zone of inhibition against the minimum zone of inhibition (set as 1) within the same category, such that E_R = Zone $_{max}$ / 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 Hand and Body Wash Gels against *E. coli* is 'HiGeen Hand and Body Wash Gel.' The number of experimental observations is n = 3

4. Discussion

4.1. Infection Control and Microbial Analysis

This study has investigated the laboratory patterns of microbial growth of saprophytic and pathogenic Gram-negative bacteria in response to disinfectants and various sterilants. The variations observed underscores the significance of using appropriate concentrations for specified periods of time, under controlled conditions, thus jibing with previously reported results pertaining to Gram-positive bacteria [1,2,5,8,13,15,21-29,33,48]. I this regard, the EPA has recently published a consortium on public health issues relating 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]*.' 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 [1-6].

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 steriliants 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 [1,2,51,52,53,54,55].

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].

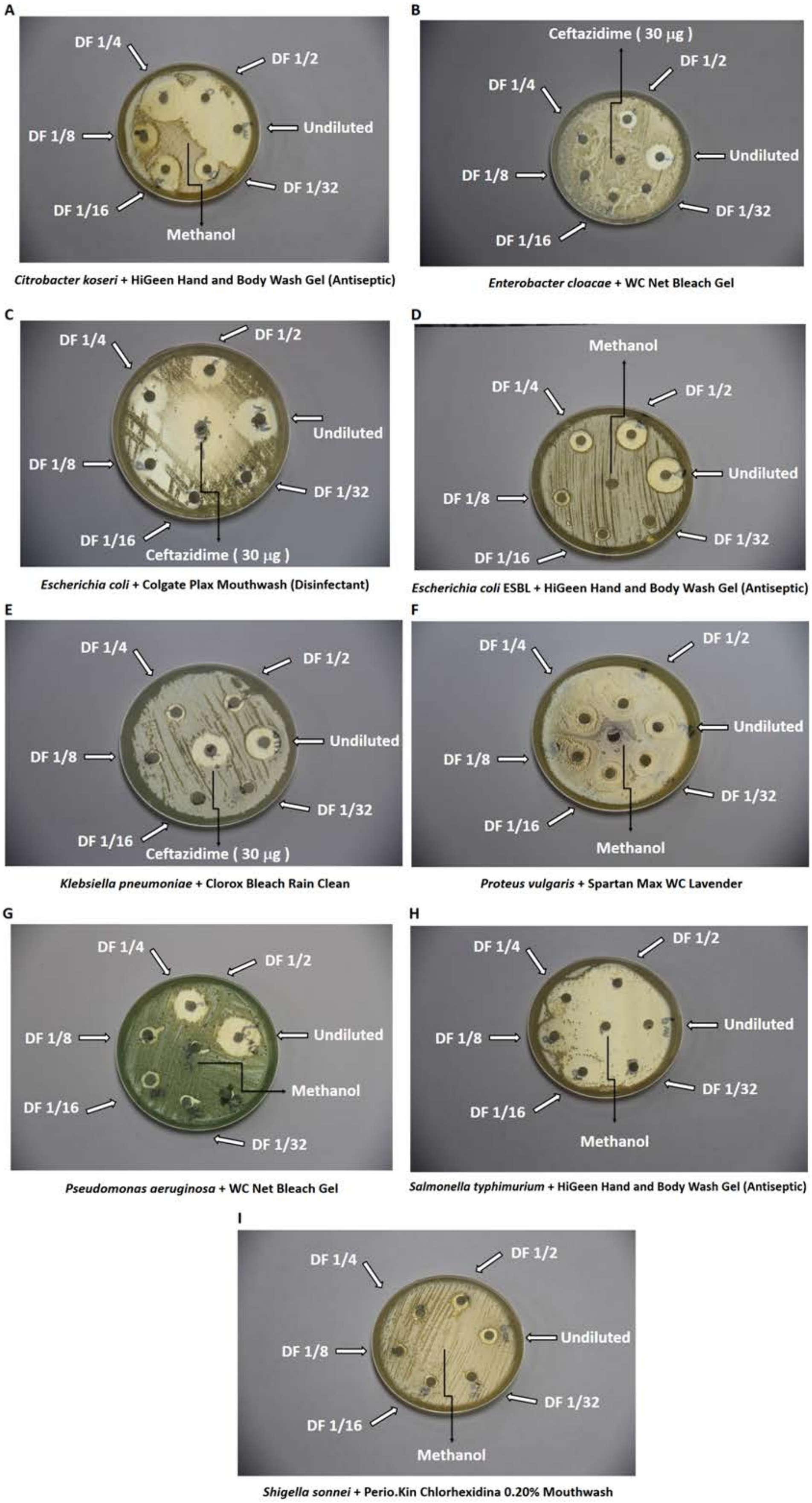

Figure 14. Typical microbial growth of gram-negative bacteria in the presence of commercially available disinfectants and antiseptics in culture. (**A**) *Citrobacter koseri* + 'HiGeen Hand and Body Wash Gel' at various concentrations (undiluted, 1/2, 1/4, 1/8, 1/16, and 1/32 + negative control, methanol; or positive control, ceftazidime (30 μg)), noting zones of inhibition. (**B**) *Enterobacter cloacae* + 'WC Net Bleach Gel'. (**C**) *Escherichia coli* + 'Colgate Plax Mouthwash'. (**D**) *Escherichia coli* ESBL + 'HiGeen Hand and Body Wash Gel'. (**E**) *Klebsiella pneumoniae* + 'Clorox Bleach Rain Clean'. (**F**) *Proteus vulgaris* + 'Spartan Max WC Lavender'. (**G**) *Pseudomonas aeruginosa* + 'WC Net Bleach Gel' (Note the typical greenish color of *P. aeruginosa*). (**H**) *Salmonella typhimurium* + HiGeen Hand and Body Wash Gel'. (**I**) *Shigella sonnei* + Perio.Kin Chlorhexidina 0.20% Mouthwash'. The number of experimental observations is n = 3. DF = Dilution factor

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 [1,2,3,4,5,116-125]:

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*."

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 [1,2]. The standardized methods of sterilization and disinfection, according to APIC guidelines for infection control practice are subsequently given [1,2].

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 [1,2,3,4,5,141-155]. 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-negative (and gram-positive) 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]:

i) *Citrobacter koseri* – Class D > Class A > Class B > Class C.

ii) *Enterobacter cloacae* – Class D > Class A > Class B > Class C.

iii) *Escherichia coli* – Class D > Class A > Class B > Class C.

iv) *Escherichia coli ESBL* – Class D > Class A > Class B > Class C.

v) *Klebsiella pneumoniae Escherichia coli* – Class D > Class A > Class B > Class C.

vi) *Proteus vulgaris Escherichia coli* – Class D > Class A > Class B > Class C.

vii) *Pseudomonas aeruginosa* – Class B > Class C > Class D > Class A.

viii) *Salmonella typhimurium* – Class D > Class A > Class B > Class C.

ix) *Shigella sonnei* – Class D > Class A > Class B > Class C.

Importantly, the first study that investigated the use of disinfectants at home was presented in 1978 [2]. Thereafter, an astronomical number of references, herein 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-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*. 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, doorknobs 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.

4.6. Inflammatory and Anti-inflammatory 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 antisepsis, 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-κB (NF-κB); 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 outermost 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].

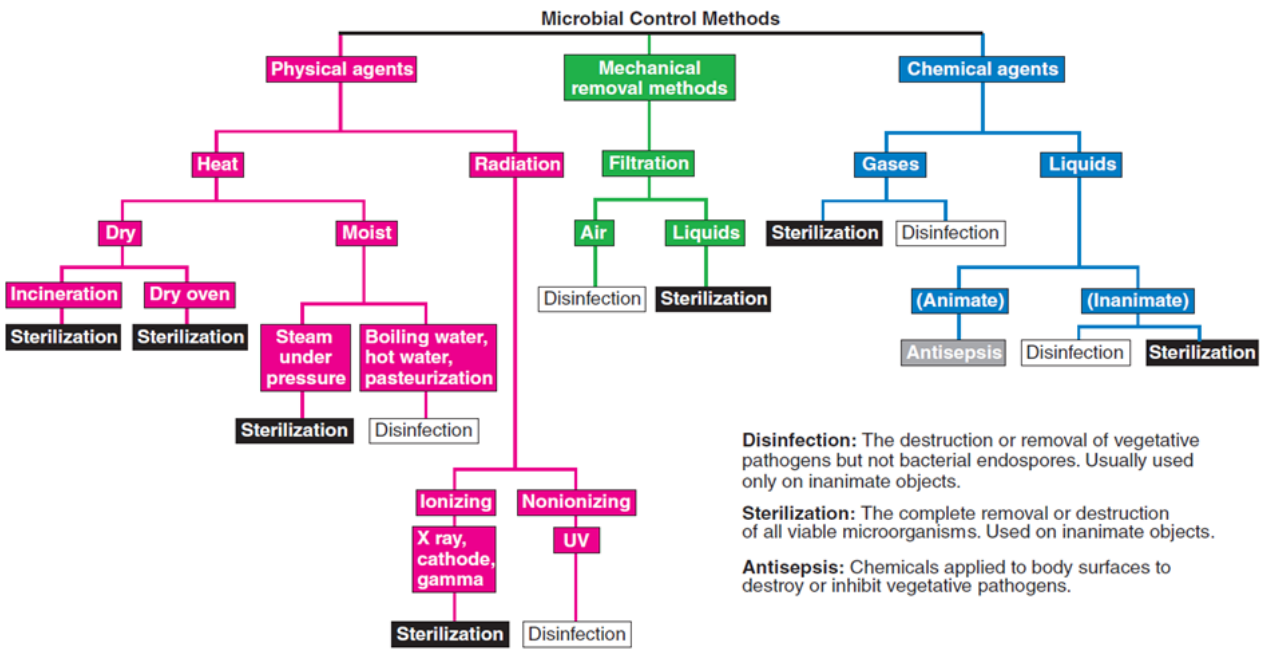

Figure 15. An overview schematic showing microbial infection control. (Adapted, courtesy of Talaro, Kathleen P., *Foundations in Microbiology*, 9th Edition, 2015. McGraw-Hill Education, USA.)

Interestingly, 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). An overview schematic showing microbial infection control is given in Figure 15.

5. Conclusions and Prospects

The present wide spectrum study has meticulously examined the 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 laboratory verified results bolster the common observations that commercially available household products are in fact 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 [1-5,25-30,45-62,91-105,116-132,175-182,198-221]. 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; Carbolic 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-κB, NF-κB; 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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