Readers Insight
Publisher of open access books and journals
PJMLS OPEN ACCESS
Print ISSN: 2707-4471. Online ISSN: 2707-448X

Research Article	Pak-Euro Journal of Medical and Life Sciences
DOI10.31580/acwt5254	Copyright © All rights are reserved by Corresponding Author
Vol. 8 No. Special 3, 2025-26: pp. S259-S268	
www.readersinsight.net/pjmls	**Revised:** March 25, 2026 **Accepted:** March 27, 2026
Submission: December 29, 2025	**Published Online:** March 31, 2026

ANTIBACTERIAL ACTIVITY OF DISHWASHING DETERGENTS AGAINST FOODBORNE PATHOGENS: A LABORATORY BASED EVALUATION

Ahmed Abbas[1], Ali Zaman[1], Muzdalfa Liaquat[1], Maaz Khan[1], Kiran[1], Ibrahim Arif[1], Tuba Aftar[1], Marium Tufail Rao[2], Sufyan Ali[3], Atiya Rabbani[4], Muhammad Salman Khalid[1, 2*]

[1]**Department of Allied Health Sciences,** IQRA University Chak Shehzad Campus, Islamabad, Pakistan
[2]**Department of Biomedical Engineering and Sciences,** School of Mechanical and Manufacturing Engineering, National University of Sciences and Technology, Islamabad, Pakistan
[3]**Institute of Molecular Biology and Biotechnology,** Bahauddin Zakariya University, Multan, Pakistan
[4]**Department of Allied Health Sciences,** National University of Medical Sciences, Rawalpindi, Pakistan

***Corresponding Author:** Muhammad Salman Khalid. E. mail: mkhalid.bms22smme@gmail.com

Abstract

Kitchen sponges are essential cleaning tools used in almost every household; however, their porous structure and moisture-retaining ability make them an ideal environment for bacterial growth. This study aimed to evaluate the antibacterial efficacy of three commonly used household dishwashing detergents against Escherichia coli ATCC 8739, Salmonella Typhi ATCC 19430, and Staphylococcus ATCC 25923. The antibacterial activity was determined using the well diffusion method, while the Minimum Inhibitory Concentration (MIC) was evaluated using the broth macro-dilution technique. The results demonstrated that all tested detergents showed antibacterial activity against Staphylococcus aureus (zones of inhibition up to 38.0 mm) but failed to inhibit the growth of Escherichia coli and Salmonella Typhi under the tested conditions. MIC analysis revealed that a detergent concentration of 10% was required to inhibit bacterial growth, which is approximately 66 times higher than the typical household concentration (0.15%). Agar well assay validates the effects of all three detergents against only one bacterial species under observation, S. aureus (38.0 ± 10.58 mm) but there was no significant activity against S. typhi or E.coli. Surprisingly, Minimum Inhibitory Concentration (MIC) testing revealed that inhibiting bacterial growth required a detergent concentration of at least 10%, which far exceeds the standard household dilution of 0.15%. This discrepancy proved statistically significant ($p = 0.0027$) across both gram-positive and gram-negative bacteria, demonstrating that everyday dishwashing detergents fall short of effectively clearing pathogens from kitchen sponges. These findings further negate the antibacterial claims of commercially available detergents and suggest a strong need for subsequent disinfection methods such as increasing the concentration of active ingredients and to regularly replace the sponges (2-4 days).

Keywords: *Antibacterial activity, Escherichia coli, Household detergents, Kitchen sponge, Minimum inhibitory concentration, Salmonella typhi, Staphylococcus aureus*

INTRODUCTION

Food safety remains one of the major public health concerns worldwide, and the domestic kitchen plays an important role in preventing foodborne illnesses. Among all kitchen cleaning tools, kitchen sponges are considered one of the most heavily contaminated items because they continuously retain moisture, food particles, and organic matter that support bacterial survival and multiplication (1, 2). Due to their porous structure, microorganisms can easily colonize the sponge and survive for prolonged periods, increasing the possibility of cross-contamination between utensils, food, and kitchen surfaces. In context of Pakistani communities, the emergence of drug-resistant *Salmonella typhi* (XDR) has further complicated the treatment of typhoid fever with more than 10,000 per year cases registered across independent laboratories and hospitals and as the kitchen sponges have been studied to facilitates the growth of this specific bacterium, there is a dire need to evaluate the efficiency of common household detergents that are used for eliminating this bacterium from the utensils (3, 4).

Contaminated kitchen sponges are studied to provide a safe place for a lot of distinct pathogenic strains, to grow rapidly, and the most highlighted ones are *Staphylococcus aureus, Escherichia coli and Salmonella typhi.* These pathogenic microbes are associated with a lot of diseases ranging from mild gastroenteritis, food poisoning to acute typhoid fever (5). Problems arise when these sponges are used repeatedly without a sort of check and balance (adequate sanitation) as these serve as a primary vector for cross-contamination of bacterial species on utensil and kitchen surfaces. While households rely heavily on the commercially available dishwashing detergents for the removal of organic residue and grease, the efficacy of these detergents against foodborne microbial species is yet to be explored, despite the antibacterial claims of these detergents (6). In addition to this, the porous and complex sponge structure renders the bacterial species towards the standard chemical exposure by developing a protective matrix. Consequently, this hinders the routine dishwashing to achieve lethal concentration of detergent or the proper contact time required to disrupt the bacterial colonies on kitchen sponges (7).

Gram-negative bacteria such as Escherichia coli and *Salmonella typhi* possess an outer membrane that provides greater resistance to surfactants and detergents, whereas Gram-positive bacteria such as *Staphylococcus aureus* are generally more susceptible (6). Consequently, scientific assessment is crucial to verify the true antibacterial efficacy of household detergents under controlled conditions. Although recent literature extensively documents the microbial populations residing in kitchen sponges, surprisingly few studies have determined the active concentrations needed to kill or inhibit these specific pathogens. A lot of commercially available detergents claims the efficient antibacterial activity of their products, but still these claims are rarely tested in real world scenarios (the concentration that is required to inhibit or to kill these foodborne pathogens (8).

The present study was conducted to evaluate the antibacterial efficacy of three commercially available household dishwashing detergents against *Escherichia coli* ATCC 8739, *Salmonella typhi* ATCC 19430, and *Staphylococcus aureus* ATCC 25923. Antibacterial activity was assessed using the well diffusion method, while the Minimum Inhibitory Concentration (MIC) was determined using the broth macro-dilution method. By quantifying the critical gap between consumer-dilution practices and actual bactericidal thresholds, the findings of this study will contribute to a better understanding of the effectiveness of household detergents and provide useful recommendations for improving kitchen hygiene practices and reducing bacterial contamination.

MATERIALS AND METHODS

STUDY DESIGN

This experimental laboratory-based study was conducted to evaluate the antibacterial efficacy of commonly available household dishwashing detergents against foodborne bacterial pathogens associated with kitchen sponges. The study was carried out in the Microbiology Laboratory, Department of Medical Laboratory Technology, Iqra University, Islamabad. All the experimentation was conducted in the microbiology lab (BSL-2) in Department of Microbiology at Islamabad Diagnostic Centre, Islamabad (F-8 branch). Guidelines for proper safety were strictly followed, and no ethical approval was deemed necessary as the study excludes any type of human or animal subjects.

BACTERIAL STRAINS

Three American Type Culture Collection (ATCC) reference strains were used in this study including, *Escherichia coli* ATCC 8739, *Salmonella typhi* ATCC 19430, and *Staphylococcus aureus* ATCC 25923. The bacterial cultures were maintained under appropriate laboratory conditions and standardized to 0.5 McFarland turbidity standards before antimicrobial testing to ensure uniform inoculum concentration.

HOUSEHOLD DETERGENTS

Three commercially available household dishwashing detergents were selected based on their popularity in the local market. For research purposes, the detergents were coded as Detergent A, Detergent B, and Detergent C to avoid commercial bias and to maintain investigator blinding during data analysis.

Further confidentiality was maintained by keeping the researchers unaware of the products until the complete recording to all experimental data. Selection criteria for all three detergents prioritized formulation methods, price ranges, and claimed antibacterial activity. The selected detergents were purchased from the local superstore one week before the commencement of research. To replicate real-world consumer scenarios, the products were tested without any modifications. The desired concentrations of fresh working solutions were prepared daily by diluting the detergents into sterile distilled water and as these formulations are designed for direct use, their pH and concentration of active ingredients were not modified either.

AGAR WELL DIFFUSION ASSAY

The anti-bacterial activity of detergents was determined through the agar well diffusion method. Mueller-Hinton agar plates were prepared according to standard microbiological protocols. The bacterial suspensions were evenly distributed on the surface of the agar medium through sterile cotton swabs. The 6 mm diameter wells were created with the help of sterile cork borer and 50 µL of each detergent was placed into respective wells. Sterile distilled water was used as a negative control along with an antibiotic disc (Ciprofloxacin, 5 µg) as a positive control to ensure the validity and efficacy of the assay under consideration. The tubes were incubated aerobically at 37°C for 24 hours after which the diameters (millimeter) of zones of inhibition were measured, showing no visible turbidity. All the experiments were conducted in triplicate to ensure precision and reliability (9).

MINIMAL INHIBITORY CONCENTRATION (MIC)

The minimal inhibitory concentration (MIC) of the detergents was determined through broth macro-dilution technique. Two-fold serial dilution of the detergents was done in Mueller-Hinton broth and to efficiently validate the findings, the concentration of detergents during dishwashing was calculated based as per the standard practices in households. A typical concentration of 5-10mL detergent was diluted in 5 liters of distilled water to achieve the estimated concentration of (0.2% v/v).The standardized bacterial inoculum was added in each test tube after which the tubes were incubated at 37°C for 24 hours (10). After the preparation of standard, a working solution of 10% (v/v) was utilized to look for the bacterial inhibition, given that lower concentrations of detergents are likely to contribute towards effective results as per the previous studies.

DATA ANALYSIS

All the experimental assays including agar well diffusion and minimum inhibitory concentration (MIC) were performed in triplicates. Quantitative data for the zones of inhibition are expressed as mean ± standard deviation, while MIC values were recorded as the lowest inhibitory concentration. Statistically significant differences between the antibacterial activities of the detergents (individual and combined) were assessed using analysis of variance (ANOVA), followed by Tukey's post-hoc test for pairwise comparisons. Prior to this parametric analysis, Shapiro-Wilk test was used for assessing the normality of distribution in data points of zone of inhibition. Meanwhile, the association between susceptibility profiles (bacterial sensitivity vs resistant) and cell wall characteristics of all the three strains (gram-negative vs gram-positive) was evaluated by Pearson's Chi Square (x^2) categorical analysis. All the tests were conducted using the latest version of IBM SPSS version 26.0 and Microsoft excel. For the tests to be statistically significant, p value less than 0.05 was set as a threshold.

RESULTS

The antibacterial activity of the three selected household dishwashing detergents was evaluated against *Escherichia coli* ATCC 8739, *Salmonella typhi* ATCC 19430, and *Staphylococcus aureus* ATCC 25923 using well diffusion and broth macro-dilution methods. The results demonstrated clear differences in susceptibility between the tested strains. In the agar well diffusion assay, all three detergents produced clear zones of inhibition exclusively against *Staphylococcus aureus* (Table I), with the largest zone observed for

detergent B (38.0 ± 3.5 mm), followed by detergent A (32.5 ± 2.1 mm) and detergent C (30.0 ± 2.8 mm). In contrast, no inhibition zones were observed against *Escherichia coli* and *Salmonella typhi*, suggesting resistance to the tested detergents under laboratory conditions.

Table I. Antibacterial activity of individual household detergents against selected bacterial strains

Bacterial strain	Gram reaction	Zone of inhibition (mm, Mean ± SD)			
		Detergent A	Detergent B	Detergent C	Result
Staphylococcus aureus (ATCC 25923)	Gram-positive	32.5 ± 2.1	38.0 ± 3.5	30.0 ± 2.8	Sensitive
Escherichia coli (ATCC 8739)	Gram-negative	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	Resistant
Salmonella typhi (ATCC 19430)	Gram-negative	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	Resistant

A one-way ANOVA was conducted, and it revealed no significant difference among all the three detergents, when tested against *S. aureus* ($p = 0.423$), highlighting the comparable anti-microbial activity across the commercial brands. Conversely, all the individual detergents evaluated failed to produce any inhibition zones against *S. Typhi* and *E. coli*, further confirming the resistance shown by gram-negative bacteria. As schematically represented (Fig. 1), when agar plates inoculated with the respective bacterial strains were loaded with detergents A, B, and C, clear zones of inhibition appeared exclusively on plates containing *S. aureus*. In contrast, the only agar plate with clear zone of inhibition (sensitivity) was the one containing *Staphylococcus aureus* while the agar plates containing *E. coli* and *S. typhi* do not show any subsequent zone of inhibitions. Overall, all three individual detergents were effective in inhibiting the growth of *S. aureus* under evaluated test conditions (10% v/v detergent concentration, 37^{0}C).

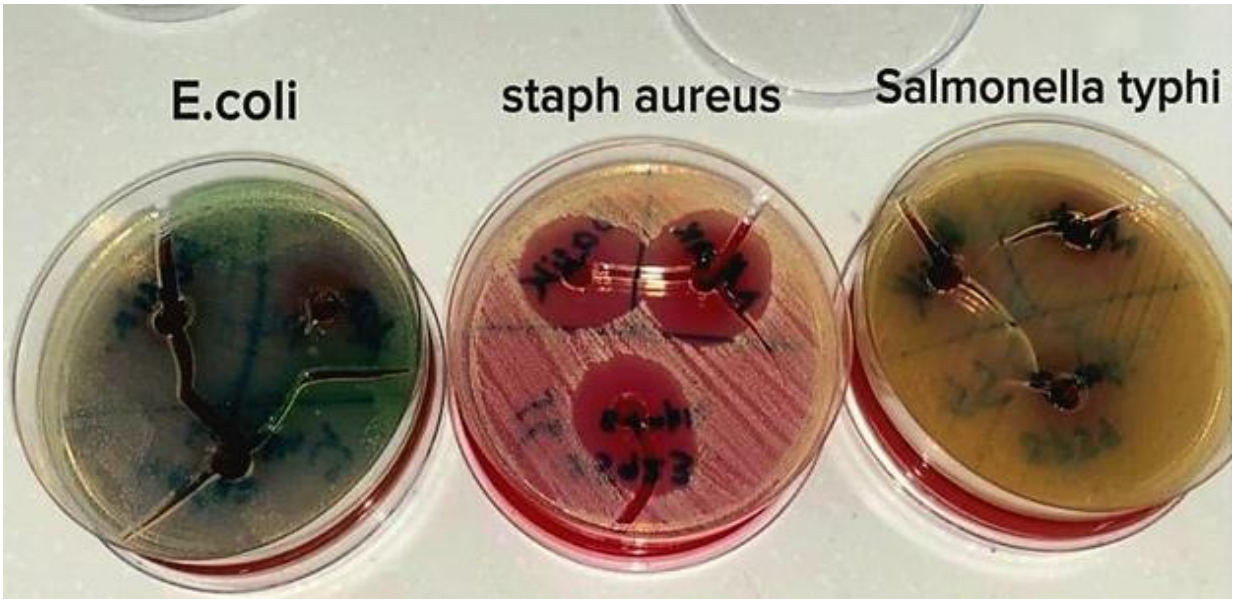

Fig. 1. Agar plates showing the inhibitory zones of the evaluated detergents against all three bacterial strains. Each Agar plate contains 3 wells with subsequent concentration of individual detergents (A, B, C). Wells having (6 mm diameter) containing 50 µL of each detergent were analyzed for zone of inhibitions (As an indication of bacterial growth). The plate containing *S. aureus* (middle) does show clear zone of inhibitions

Given the resistance of individual detergents against both gram-negative bacterial strains, we further investigated whether there are synergistic inhibitory effects when these detergents are used in combinations of two or more. The following combinations of detergents were utilized to assess the antimicrobial effects on gram-negative and gram-positive bacterial strains alike including A+B, B+C, A+C and A+B+C using the same methodology as was utilized in agar well diffusion assay for single detergents. The combination assays revealed that these mixtures produced varying zones of inhibition not only against *Staphylococcus aureus*, but also against *Salmonella typhi*. The zones produced against *S. typhi* were in fact lesser than *S. aureus* but still large enough to be seen than the individual detergents (where there were no zones of inhibition whatsoever). On contrary to this, still there were no zones of inhibition against the *E. coli*

even when the all the three detergents were used simultaneously. The quantitative results of combination assays are further demonstrated in the table below (Table II).

Table II. Antibacterial activity of combination assays against all the three strains under observation

Bacterial strains	Detergent combination	Results	Zone of inhibition (mm)
Staphylococcus aureus	A+B	Sensitive	50
Staphylococcus aureus	B+C	Sensitive	30
Staphylococcus aureus	A+C	Sensitive	34
Staphylococcus aureus	A+B+C	Sensitive	30
Salmonella Typhi	A+B	Light Zone	15
Salmonella Typhi	B+C	Clear Zone	20
Salmonella Typhi	A+C	Light Zone	15
Salmonella Typhi	A+B+C	Light Zone	17
Escherichia coli	A+B	Resistant	0
Escherichia coli	B+C	Resistant	0
Escherichia coli	A+C	Resistant	0
Escherichia coli	A+B+C	Resistant	0

To further investigate the comparative efficacy of the combination assays, a detailed and concise quantitative assessment was conducted across the pathogenic strains of gram-positive and negative bacterial strains. The combination assays also showed variable antimicrobial activity against *S. aureus* (Fig. 2a) and a moderate susceptibility towards *S. typhi* (Fig. 2c) with the mean zone of inhibition of 36.0 ± 9.52 mm and 16.75 ± 2.36 mm respectively. But still, no antibacterial activity was observed against the gram-negative bacterium *E. coli* across all the observed replicates **(**Fig. 2b). The results clearly demonstrated that the combination of tested detergents was able to effectively inhibit the growth of gram-positive bacterium *S. aureus*, but their efficacy against the gram-negative bacterial strains was remarkably constrained. This differential antimicrobial profile is most likely to be attributed to the outer barrier of lipopolysaccharides that provides an additional layer of protection against the active chemical ingredients of detergent rendering the cell resistance against surfactant driven lysis. Descriptive statistics of zone of inhibition, including mean zone of inhibition, ranges, median values and standard deviations are presented in (Table III).

Table III. Quantitative summary of zone of inhibition (Mean ± Standard deviation (mm)) for detergent combinations

Organism	Zone of inhibition	Range (mm)	Median (mm)
S. aureus	36.0 ± 9.52 mm	30–50	34
S. Typhi	16.75 ± 2.36	15–20	16
E. coli	0.0	0	0

The minimum inhibitory concentration (MIC) of all the three tested detergents was determined using the method of broth macro-dilution due to its ability to determine the quantitative baseline (minimum concentration) for detergents potency against bacterial strains, across a range of 10% to 0.078%. At 10% concentration, all detergents showed same MIC value with no significant differences and surprisingly, when the concentrations were reduced to 5%, 2.5%, 1.25% till 0.078, the bacterial strains showed visible growth. This further points out that this lower concentration failed to meet the minimum threshold for bacterial inhibition. To further contextualize these findings, the MIC values were comparatively analyzed against the detergent concentration, used in routine dishwashing in households.

These findings highlight the substantial discrepancy that the household concentrations during dishwashing are much lower than the actual concentrations required inhibit/kill the foodborne microbial strains. Apart from this, these findings challenge the manufacturers claim (kills 99.9% microbes) and urge the use of more active ingredients in the commercialized detergents. Based on the standard household practices 5-10 mL detergent in 5 L of tap water, the approximate used concentration is 0.15%-0.25% (v/v) which is roughly 40- to 66-fold lower than the MIC (10%) determined in this study **(**Table 4).

The comparative analysis among antibacterial activity of all the three detergents against *S. aureus* was assessed using ANOVA. The results revealed no statistical difference among the tested detergents ($F = 1.02$, $p = 0.423$), highlighting the comparable antibacterial activities across all the brands. Furthermore, this comparable finding reveals that when detergents are used individually, their antibacterial efficacy was

independent of the brand at tested concentrations. In addition to this, antibacterial activities of the combination assays (A+B, B+C, A+C, and A+B+C) against *S. aureus* were also evaluated by one-way ANOVA and same results were observed as there was no significant difference (F = 2.14, p = 0.178) between these combinations as well. However, descriptive statistics highlighted the highest zone of inhibition (50 mm) produced by the detergent combination A + B suggesting an additive antimicrobial effect suggesting an additive antimicrobial effect that warrants further study with a larger sample size.

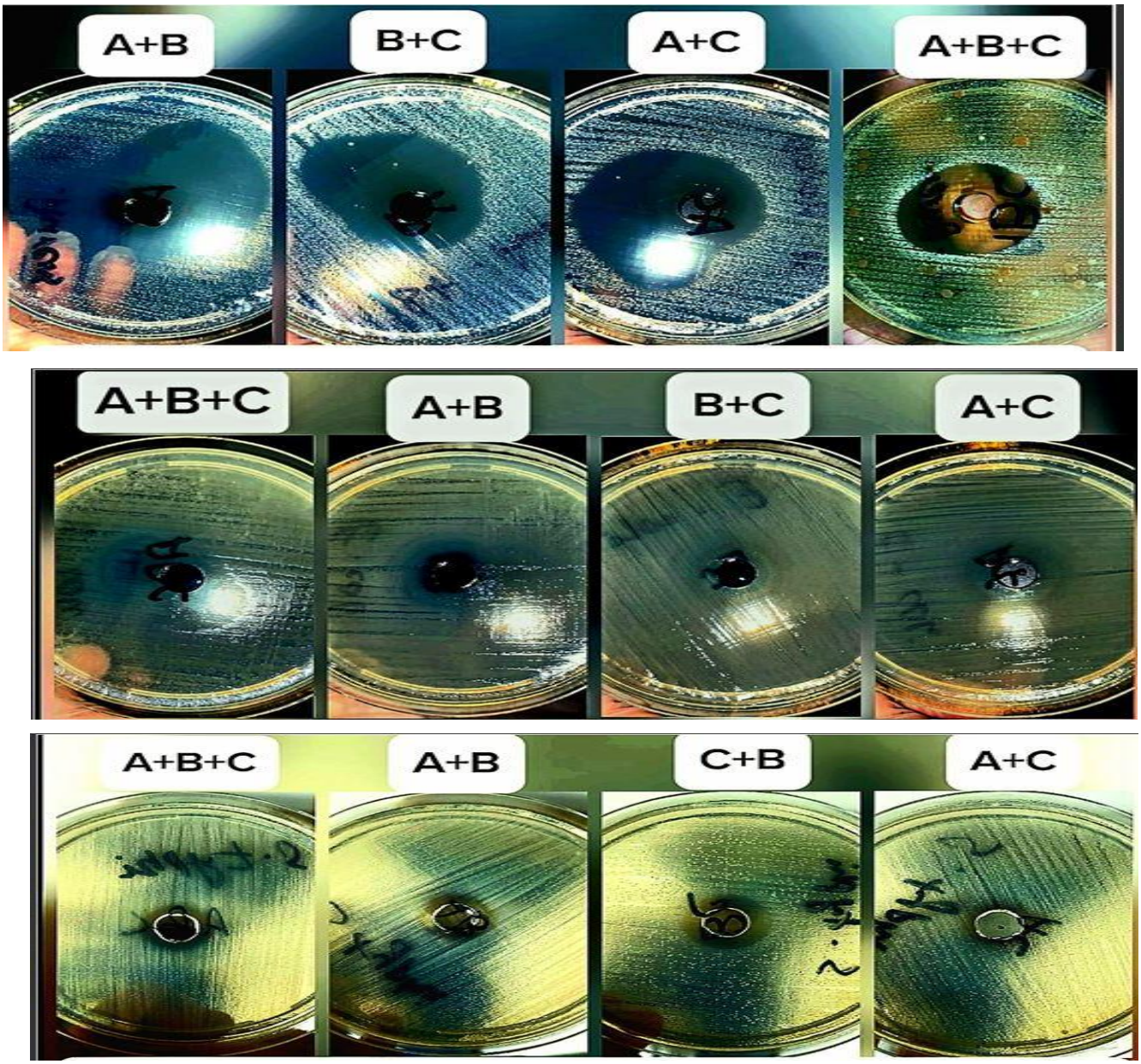

Fig. 1. Zone of inhibition assay showing the effect of detergent combinations on three bacterial strains. Wells contained A+B, B+C, A+C, and A+B+C mixtures. *Staphylococcus aureus* **(2a)** exhibited the largest zones of inhibition, indicating highest sensitivity. *Salmonella typhi* **(2c)** showed small but clear zones with mixed detergents, suggesting enhanced activity compared to single detergents. *Escherichia coli* **(2b)** showed no bacterial inhibition (clear zones), indicating resistance

To further validate the association between susceptibility to detergent treatments and cell wall characteristics, a Chi-square (χ^2) analysis was performed. The analysis revealed that gram-positive bacteria (*S. aureus*) were relatively more susceptible to household detergents than gram-negative bacterium (*E. coli* and *S. typhi*), individual as well as combination assays, with an association of $\chi^2 = 9.00$ (df =1, p = 0.0027). This means that the observed difference in effectiveness between the gram-positive and gram-negative bacteria is very unlikely to be due to chance and strongly suggests that the cell wall structure is a key factor in a detergent's effectiveness. Moreover, one-way ANOVA was subsequently performed to compare the mean zone of inhibition against the tested bacterial strains. The analysis revealed a statistically significant outcome with an F value of 98.45 and $p < 0.001$ and to further validate the F statistics, a post-hoc analysis

(HSD) was conducted to check the susceptibility profile. The analysis confirmed that *S. aureus* exhibited a significantly higher susceptibility (mean zone of 36.0 ± 9.52 mm) than *S. typhi* (16.75 ± 2.36 mm) and E. coli (0.0 ± 0.0 mm) with a p value of 0.0031 and 0.0025, respectively. Furthermore, this statistically significant difference also confirms that the detergent combinations were far more effective against the gram-positive *S. aureus* than against the gram-negative *S. Typhi* (Table V).

Table IV. Comparison of the minimum inhibitory concentration (MIC) of tested detergents against bacterial strains versus routine household usage concentrations. MIC was determined by macro-dilution methods, and the subsequent concentration was set within the range of 10% to 0.078%. Growth was observed at concentrations below 10% and all the detergents revealed identical antimicrobial activity. Household concentrations were studied to be 66.7 times lower than MIC

Bacterial strain	Detergent	MIC (% v/v)	Growth at 5% and below?	Household use concentration	Fold-difference	Efficacy in household concentration
Staphylococcus aureus	A	10	Yes	0.15%–0.25%	66.7-fold lower than MIC	Ineffective
Staphylococcus aureus	B	10	Yes	0.15%–0.25%	66.7-fold lower than MIC	Ineffective
Staphylococcus aureus	C	10	Yes	0.15%–0.25%	66.7-fold lower than MIC	Ineffective
Salmonella Typhi	A	10	Yes	0.15%–0.25%	66.7-fold lower than MIC	Ineffective
Salmonella Typhi	B	10	Yes	0.15%–0.25%	66.7-fold lower than MIC	Ineffective
Salmonella Typhi	C	10	Yes	0.15%–0.25%	66.7-fold lower than MIC	Ineffective
Escherichia coli	A	10	Yes	0.15%–0.25%	66.7-fold lower than MIC	Ineffective
Escherichia coli	B	10	Yes	0.15%–0.25%	66.7-fold lower than MIC	Ineffective
Escherichia coli	C	10	Yes	0.15%–0.25%	66.7-fold lower than MIC	Ineffective

Table V. Statistical analysis comparing antimicrobial activity against detergent types, treatment conditions and three bacterial strains. A one-way analysis of variance was conducted to evaluate the significance in zone of inhibition along with Tukey' post-hoc test (pairwise comparison). The association between bacterial type and susceptibility profiles was analyzed using Chi-square analysis with significance set at $p < 0.005$ (triplicate experiment)

Comparison	Test Used	Test Statistic	p-value	Significance
Individual detergents (A, B, C) vs. *S. aureus*	One-way ANOVA	F = 1.02	0.423	Non-Significant
Detergent combinations vs. *S. aureus*	One-way ANOVA	F = 2.14	0.178	Non-Significant
Gram-type vs. Susceptibility	Pearson's Chi-square	$\chi^2 = 9.00$, df = 1	0.0027	Significant
Bacterial strain comparison (combined treatments)	One-way ANOVA	F = 98.45	<0.001	Significant
S. aureus vs. *S. Typhi*	Tukey's HSD	Mean diff = 19.25 mm	0.0031	Significant
S. aureus vs. *E. coli*	Tukey's HSD	Mean diff = 36.0 mm	0.0025	Significant
S. Typhi vs. *E. coli*	Tukey's HSD	Mean diff = 16.75 mm	0.0043	Significant

DISCUSSION

The current study points out crucial insights into the antimicrobial effects of three distinct dishwashing detergents on dishwashing sponges, commonly used across various cities in Pakistan. The results revealed a complex disparity between the antimicrobial efficacies under standard concentrated laboratory conditions highlighting important implications for improving kitchen hygiene practices.

One of the most critical findings of this study is the significant difference between the gram-negative and gram- positive bacterium towards tested household detergents. *Staphylococcus aureus (*gram-positive bacterium, strain ATCC 25923) showed excellent susceptibility towards the tested with varying zone of inhibitions against individual detergents (30.0–38.0 mm) and the combination assays (30–50 mm). On the contrary, gram- negative bacterial strains of *Escherichia. coli* (strain ATCC 8739) and *Salmonella typhi* (strain ATCC 19430) exhibited complete resistance against the individual detergents at varying concentrations and only a moderate susceptibility profile against certain detergent combinations was observed in *S. typhi* with zone ranging from 15-20 mm but still the *E. coli* showed complete resistance. This distinct susceptibility profile of *E. coli* is best described by the presence of an outer membrane of lipopolysaccharides (LPS) (11, 12) and phospholipids conjugates, absent in gram positive bacterial strains like that of *S. aureus* (porous peptidoglycan layer allows easier penetration of detergents) (13). But despite the gram-negative profile of *E. coli* and *S. typhi,* the observed differential susceptibility may be because of variations in the composition of lipopolysaccharides and structural protein. Compared to *E. coli, S. typhi* possesses a complex and larger O-polysaccharide chain (antigenic chain on cell membrane) that renders it vulnerable to surfactant-induced disruption. These membrane integrated molecules act as barrier against surfactants; hydrophobic compounds (lipid A acylation) and several distinct antimicrobial compounds present in detergents.

This association of antimicrobial susceptibility between gram-positive and gram-negative bacterial strains was further validated by Pearson's Chi-square test ($p = 0.0027$). Moreover, the resistance profile of *E. coli* against all the tested detergent combinations suggests the prevailing robust resistance mechanism, including, but not limited to alteration in membrane permeability (expression of porin proteins) , formation of bacterial biofilms and upregulation of efflux pumps that actively expels the antibacterial moieties out of the cell (14, 15).

Furthermore, a synergistic antibacterial effect was observed against *S. aureus* after treating the strain with detergents combination. While there was no zone of inhibition observed with individual detergents, the combination of detergents B+C revealed a clear and distinct zone of 20 mm. These interesting findings suggest that different detergents do contain active complementary agents that may further increase the susceptibility profile of microbial strains towards the tested detergent. This integrated effect is highly attributed to the presence of chelating agents, types of surfactant or other active ingredients in commercial formulations (6). The combination B+C subsequently outperformed other detergent combinations (A+C, A+B, A+B+C) against *S. typhi,* indicating that these two combinations contain active complementary ingredients surpassing other combinations utilized in this study. Nonetheless, the lack of antimicrobial activity against *E. coli* underscores the remarkable resistance profile of this species and the limitations of detergent-based phenomenons for inhibiting this foodborne pathogen in household.

A remarkably interesting observation was the discrepancy between determination of MIC and agar well diffusion methods. While *S. aureus* shows excellent zone of inhibitions in agar assays with sizes ranging from 30-50 mm, its minimum inhibitory concentration profile was the same as that of gram-negative bacterial strains (*E. coli* and *S. typhi*). This finding highlights a critical shortcoming of agar well diffusion assay as it only provides semi-quantitative information about antimicrobial activity rather than prediction of the exact concentration required for bacterial inhibition in a liquid medium (16). The MIC assay gave us a clearer insight into the antibacterial potency as it demonstrates the concentration required for inhibition of bacterial growth. Apparently, it is clearer to state that while agar assays showed excellent activity, the high MIC (10%) reveals that effective inhibition requires concentrations far exceeding standard domestic use (17).

Another significant finding of this study is the discrepancy between the concentration of detergent used in households (0.15%-0.25%) and the observed MIC value (10%), highlighting the fact that the

household concentration of detergents is subsequently lower than the required concentration for bacterial inhibition (18). This finding correlates with public health and critical kitchen hygiene practices as, despite the antibacterial claims (kills 99.9% microbes) of these commercial brands, our results severely contradict these claims as there was no elimination of gram-negative strains. These results are of great concern in local communities where extensively drug-resistant strains of *Salmonella typhi* pose severe health-related challenges (4). Kitchen sponges are known for providing a favorable niche for bacterial growth and if left untreated, can act as an active reservoir for resistant microorganisms (8, 19). Furthermore, our findings not only challenge the antimicrobial claims of these tested commercial brands but also support previously reported studies questioning the antibacterial efficacy of common household detergents (20, 21).

The laboratory conditions in the current study reflect only a simplified model, not a clear picture of complex household environment. Typical household dishwashing occurs within the temperature of 40-50^{0}C along with food debris and mechanical agitation, which could influence the antibacterial efficacy of detergents. Therefore, these findings can only be considered as a conservative estimate of antibacterial profiles of detergents under laboratory-based settings (37^{0}C). The inconsistency between the household concentrations (0.15-0.25%) and observed MIC (10%) suggests that routine dishwashing may not be able to effectively inhibit bacterial growth in the absence of elevated temperatures and gentle mechanical agitation.

CONCLUSION

This study demonstrates that commercial dishwashing detergents show differential antibacterial activity, with gram-positive *S. aureus* being more susceptible than gram-negative *E. coli* and *S. typhi.* No formulation was effective against *E. coli,* indicating a resistance profile in this foodborne pathogen. The minimum inhibitory concentration required (10%) was far higher than routine household concentrations (0.15%–0.25%), challenging the claimed 99.9% antibacterial efficacy of these products under standard laboratory conditions. These findings are significant for Pakistani communities facing drug-resistant Salmonella outbreaks, where kitchen sponges may harbor such pathogens. Overall, household detergents alone cannot guarantee microbiological safety, highlighting the need for additional disinfection strategies and regular sponge replacement to reduce foodborne illness risks.

Limitations & prospects:

Despite the positive outcomes, several limitations of the current study should be acknowledged. First, the evaluation was solely conducted under standard laboratory conditions using planktonic culture of bacterial strains; real world efficacy of detergents may show variations due to hardness of water, temperature fluctuations, formation of bacterial biofilms and food residues. Second, only three bacterial strains and three detergent formulations were used which further limits the generalizability of our findings to other wild-type bacterial isolates or other commercially available products. Finally, MIC was conducted for the determination of bacterial growth rather than assessing the mortality profile, skipping bactericidal against bacteriostatic characteristics. Future studies should evaluate a larger range of commercially available detergent products under household scenarios rather than laboratory conditions by utilizing time-kill assays combined with Minimum bactericidal concentration assay (MBC).

Conflict of interest:

The authors declare that they have no competing interest.

Acknowledgments:

The authors share their deepest gratitude to Brigadier Doctor Gohar Zaman (MBBS-Consultant Microbiologist) and Ms. Mehwish Muneer (Senior Microbiologist) at IDC-F8 for their valuable support and guidance throughout the entire study.

Author's Contributions:

AA, AZ & IM Conducted the experimental work and data collection; MSK, SA & TA Supervised the study and reviewed the manuscript; MSK, MTR & KQ Performed data analysis and interpretation; AA, AZ & IM

Assisted in laboratory assays; MSK Participated in manuscript drafting and editing; AR Participated in manuscript review & editing.

References:

1. Marotta SM, Giarratana F, Calvagna A, Ziino G, Giuffrida A, Panebianco AJIjofs. Study on microbial communities in domestic kitchen sponges: Evidence of Cronobacter sakazakii and Extended Spectrum Beta Lactamase (ESBL) producing bacteria. 2019;7(4):7672.
2. da Silva Farias A, Akutsu RdCCdA, Botelho RBA, Zandonadi RPJIJoER, Health P. Good practices in home kitchens: Construction and validation of an instrument for household food-borne disease assessment and prevention. 2019;16(6):1005.
3. Menini A, Mascarello G, Giaretta M, Brombin A, Marcolin S, Personeni F, et al. The critical role of consumers in the prevention of foodborne diseases: An ethnographic study of Italian families. 2022;11(7):1006.
4. Qamar FN, Yousafzai MT, Sultana S, Baig A, Shakoor S, Hirani F, et al. A retrospective study of laboratory-based enteric fever surveillance, Pakistan, 2012–2014. 2018;218(suppl_4):S201-S5.
5. Kusumaningrum H, Van Putten M, Rombouts F, Beumer RJJofp. Effects of antibacterial dishwashing liquid on foodborne pathogens and competitive microorganisms in kitchen sponges. 2002;65(1):61-5.
6. Kudla M, Tewes TJ, Gibbin-Lameira E, Harcq L, Bockmühl DPJM. Hygiene Efficacy of Short Cycles in Domestic Dishwashers. 2025;13(7):1542.
7. Todd EC, Greig JD, Bartleson CA, Michaels BSJJofp. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 6. Transmission and survival of pathogens in the food processing and preparation environment. 2009;72(1):202-19.
8. De Lella A, Nappi R, Anzalone A, Nappa M, Brunetti R, Montone AMI, et al. Microbiological contamination of domestic kitchen sponges and associated hygiene practices. 2026;17:1852521.
9. Balouiri M, Sadiki M, Ibnsouda SKJJopa. Methods for in vitro evaluating antimicrobial activity: A review. 2016;6(2):71-9.
10. Clinical, Institute LS. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard: Clinical and Laboratory Standards Institute; 2015.
11. Delcour et. al AHJBeBA-P. Outer membrane permeability and antibiotic resistance. 2009;1794(5):808-16.
12. Nikaido ea. Molecular basis of bacterial outer membrane permeability revisited. 2003;67(4):593-656.
13. Silhavy eaJCSHpib. The bacterial cell envelope. 2010;2(5):a000414.
14. Gilbert P, McBain A, Rickard AJIb, biodegradation. Formation of microbial biofilm in hygienic situations: a problem of control. 2003;51(4):245-8.
15. Srey ea, Sang-Do %J Food control. Biofilm formation in food industries: A food safety concern. 2013;31(2):572-85.
16. Wiegand I, Hilpert K, Hancock REJNp. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. 2008;3(2):163-75.
17. Al-Said HM, Ashgar SSJAJoMR. A comparison of antibacterial efficacy of some household detergents available in Makkah, Saudi Arabia, against 16 ATCC bacterial strains and clinical isolates. 2020;14(8):388-94.
18. Nielsen P, Brumbaugh E, Kananen LJJoAI. Evaluation of the use of liquid dishwashing compounds to control bacteria in kitchen sponges. 2002;85(1):107-12.
19. Cardinale M, Kaiser D, Lueders T, Schnell S, Egert MJSr. Microbiome analysis and confocal microscopy of used kitchen sponges reveal massive colonization by Acinetobacter, Moraxella and Chryseobacterium species. 2017;7(1):5791.
20. Russell et.al AJJoac. Similarities and differences in the responses of microorganisms to biocides. 2003;52(5):750-63.
21. Al-Said HM, Ashgar SS. A comparison of antibacterial efficacy of some household detergents available in Makkah, Saudi Arabia, against 16 ATCC bacterial strains and clinical isolates. African Journal of Microbiology Research. 2020;14(8):388-94.