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ORIGINAL RESEARCH ARTICLE

Comparative Efficacy of Some Commonly Used Disinfectants Against Bacteria Isolated from Toilet Floors of College of Natural and Pharmaceutical Sciences, Bayero University, Kano

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ABSTRACT

Disinfectants are chemicals capable of destroying or inhibiting the growth of microorganisms on inanimate surfaces. Disinfecting toilet floors is a fundamental aspect of infection control in schools and communities. This study aimed to evaluate the effectiveness of three disinfectants against bacteria isolated from toilet floors at the College of Natural and Pharmaceutical Sciences, Bayero University, Kano. Swabs were collected using sterile cotton swabs after treatment with disinfectants. Bacterial isolates were identified through standard microbiological methods, with biochemical tests used to confirm their presence. The effectiveness of three disinfectants, Chloroxylenol, isopropyl alcohol (X), Sodium hypochlorite (Y), and 32% Phenol (Z), was tested against the isolated bacteria. Three concentrations (100%, 75%, and 50%) of each disinfectant were tested on all organisms using the agar well diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each disinfectant were determined. The organisms were also tested against some standard antibiotics. The novelty of this study lies in isolating five environmentally important bacterial species directly from toilet floor surfaces. These include *Staphylococcus aureus*, *Escherichia coli, Klebsiella sp, Bacillus sp* and *Pseudomonas sp*. The ability of these organisms to survive and grow at graded concentrations (100%, 75%, and 50%) provides new insight into their differential tolerance and potential reduced susceptibility under real-world sanitation conditions. The results showed that X was the most active among the tested disinfectants. *Bacillus sp* resist all three disinfectants tested, while *S. aureus* is the most susceptible organism. At 50% and 25% Y and Z failed to kill the organisms. The susceptibility pattern showed that S. aureus, E. coli, and Pseudomonas sp. were more susceptible to Y at rates of 73%, 64%, and 57%, respectively. At 100% concentration, all organisms exhibit the highest zones of inhibition, 25-32mm. At 50% concentration, all isolates exhibited visible growth, indicating significant loss of disinfectant efficacy at sub-optimal concentrations. MICs of all disinfectants against all isolates ranged from 6.25 µg to 12.5 µg, except for *Klebsiella sp.* and *Bacillus spp.*, which exhibited 12.5 µg. The study demonstrated that all disinfectants were active against the tested organisms, with the highest activity observed for Y. Regular surface disinfection is essential to reduce infectious diseases caused by contact with environmental pathogens.

ARTICLE HISTORY

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Disinfectant, susceptibility, toilets, microorganism, concentration

INTRODUCTION

A restroom is any building or room designed for people to use the bathroom, which usually includes integrated toilet facilities and sinks for other related washings. Droplets from flushing contain pathogens, including faecal matter, and aerosolize due to the flushing mechanism; these aerosol and faecal matter splash onto many areas of the building: door handles, toilet faucets, adjacent floors, etc. (Suen *et al.*, 2019). These surfaces are known to harbour potentially pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella sp.*, *Bacillus sp.*, and *Pseudomonas sp.*, many of which are associated with community-acquired and hospital-acquired infections (Akanbi and Olurunfemi, 2020).

Some individuals use washbasins and touch doorknobs many times, contaminating them. This is a clear indication that they serves as a source of bacterial contamination (Matini *et al.*, 2020). Therefore, microbiological disinfection is important. Regular cleaning and disinfection are therefore essential to reduce microbial load and interrupt possible transmission pathways.

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Disinfectants such as sodium hypochlorite, phenolic compounds, quaternary ammonium compounds, and alcohol-based formulations are among the most widely used in Nigerian households, schools, public restrooms, and health care facilities (Nwankwo and Offiah, 2016).

The effectiveness of these disinfectants varies based on concentration, contact time, bacterial *spp* and environmental conditions. Several studies in Nigeria have demonstrated that while some commonly used disinfectants significantly reduce bacterial contaminants, others show reduced efficacy due to improper dilution, substandard formulation, or microbial resistance, and some data show how often and easily high-contact washroom surfaces can be contaminated (Oluyege *et al*., 2015; Adegoke and Komolofe, 2018). Antibiotic-resistant microorganisms are as prevalent today in public restrooms as in many other places. *S. aureus, E. coli,* and *Bacilli* such as *P. aeruginosa,* among others.

The widespread use of antibiotics is strongly associated with the development of antibiotic-resistant bacteria. If bacteria isolated from public restrooms demonstrate resistance to antibiotics, the problem of antibiotic resistance will escalate and worsen (Kapoor *et al*., 2017). It was reported that sodium hypochlorite and alcohol possess strong bactericidal activity against *E. coli and S. aureus,* whereas some quaternary ammonium products exhibit limited effectiveness against *P. Aeruginosa,* a known disinfectant-resistant organism (Nwankwo and Offiah, 2016).

However, despite routine cleaning efforts and the use of various disinfectants, understanding the relative effectiveness of these cleaning and disinfectants, which is crucial, especially in environments with high exposure risk such as public restrooms and university toilet floors, remains uncertain. The present study aims to compare the efficacy of some commonly used disinfectants against bacteria isolated from toilet floors of the College of Natural and Pharmaceutical Sciences, Bayero University, Kano

METHODOLOGY

Study Area

This cross-sectional design study was conducted at the College of Natural and Pharmaceutical Sciences, Bayero University, Kano. The college consists of three faculties: the Faculty of Pharmaceutical Sciences, the Faculty of Life Sciences, and the Faculty of Physical Sciences. Figure 1 provides the summary of the methodology.

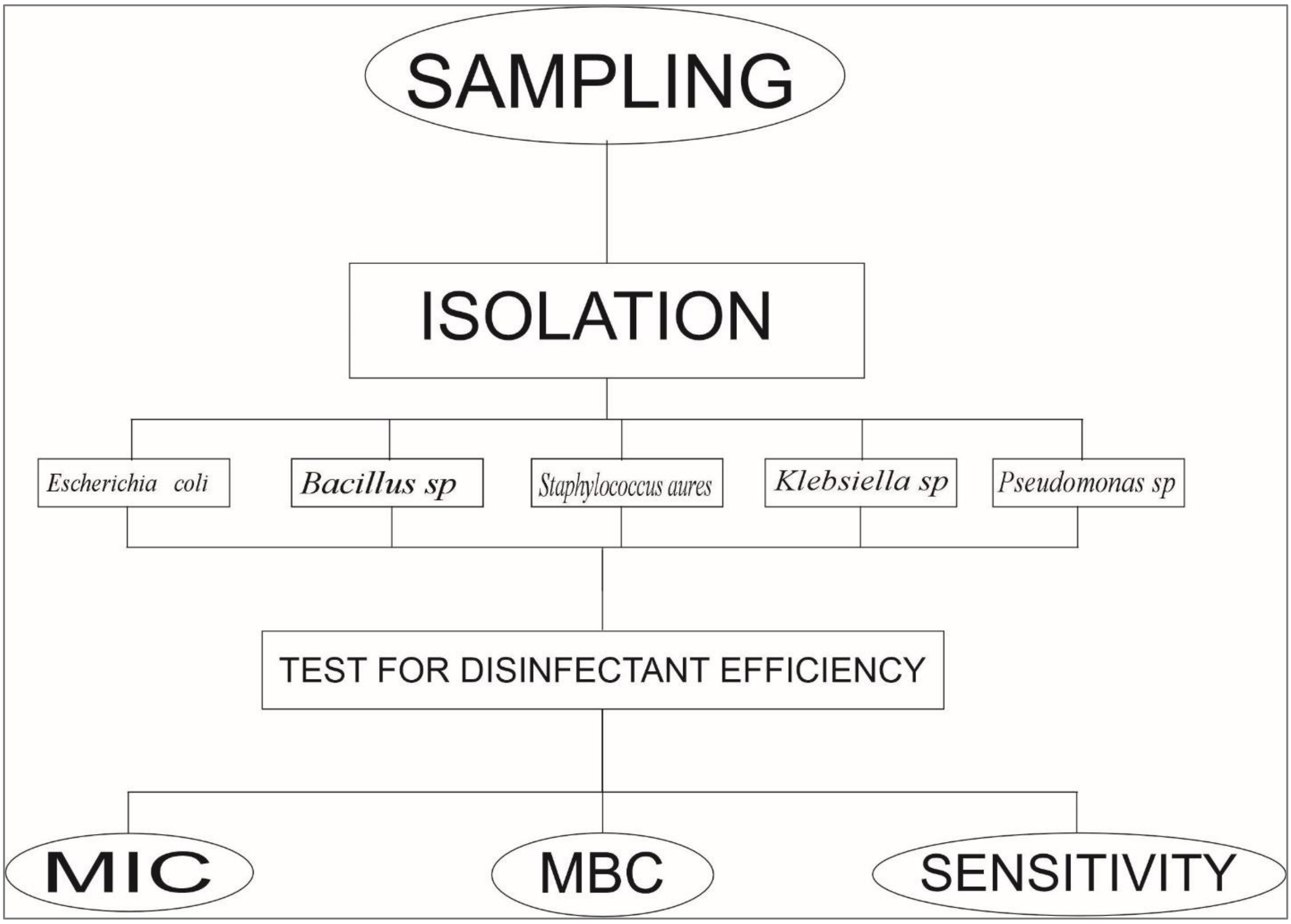

Figure 1: Flow Chart of the Study

Sample Size Determination

A sample size of 180 was considered appropriate for this study. The sample size was determined using the standard formula for estimating a population proportion at a 95% confidence level, with a margin of error of approximately ±7.3% and a conservative estimated proportion of p=0.5 to ensure maximum variability (Abdulrahman *et al*., 2025).

Thus, a sample of 180 is statistically sufficient to produce reliable estimates with acceptable precision.

$n = Z^2 \times p\ (1-p)/E^2$

Where: n= the desired sample size
Z= Normal standard distribution that corresponds to the confidence interval
p= Prevalence
E = degree of accuracy/precision expected at 0.05
$Z^2 = 1.96^2 = 3.8416$

Sample Collection

Sixty (60) samples were collected from each of the three faculties, for a total of one hundred and eighty (180), and used in the study. Sterile cotton swabs were used to collect samples from toilet floors near the toilet bowl of three faculties in the college. Samples were labelled and transported to the laboratory for bacteriological analysis.

Isolation and Purification of Isolates

Cultural, Morphological and Biochemical Characterization of the Bacterial Isolates

Bacterial isolates were identified using the serial dilution and pour plate techniques described by Cheesbrough (2017). From the stock culture in nutrient broth, 1.0 ml of the sample was aseptically pipetted into a sterile test tube containing 9.0 ml of sterile normal saline, and the mixture was mixed thoroughly. One (1.0 ml) of the dilution from each test tube (10^-1 to 10^-5) was aseptically transferred into the corresponding Petri dishes. Next, molten and cooled nutrient agar (Oxoid, England Ltd) was poured onto the plates. The plates were gently swirled and allowed to solidify at room temperature. They were then incubated at 37°C for 24 hours. Following incubation, colonies were counted and recorded as colony-forming units (CFU/ml). Gram's staining was performed according to Cheesbrough (2017) to determine Gram's reaction. Phenotypically distinct colonies were subcultured onto fresh nutrient agar and incubated at 37°C for 24 hours to obtain pure bacterial cultures. Discrete colonies, identified by their shape and surface margins, were aseptically selected and subcultured onto selective media such as Mannitol salt agar, cetrimide agar, blood agar and MacConkey agar (Oxoid, England Ltd). The following biochemical tests, as described by Cheesbrough (2020), were performed to further confirm the isolates.

Biochemical and Confirmatory Test

Catalase Test

A drop of 3% hydrogen peroxide was made on a clean glass slide. Using a wooden applicator, a colony of the test organism was brought into contact with hydrogen peroxide and observed for bubbles. The presence of bubbles indicated a positive catalase test, and the absence of bubbles indicated a negative catalase test (Khatoon *et al.*, 2022).

Coagulase Test

Two drops of distilled water were placed at the ends of a clean, grease-free glass slide. A colony of the test organism was emulsified onto each drop. To one of the suspensions, a loopful of plasma was added and gently mixed. The other suspension was left as the control. Observation of agglutination within ten (10) seconds of the addition of plasma indicated a positive coagulase test. (Cheesbrough, 2017).

Indole Test

A biochemical test performed on bacterial species to determine an organism's ability to convert tryptophan into indole. The isolate was inoculated in 5ml peptone water (tryptophan broth) and incubated at 37°C for 24-48 hours. After the incubation period, 3 drops of Kovac's reagent were added to the inoculum, shaken, and observed for a reaction. A positive reaction is indicated by the development of a red colour in the reagent layer above the broth within 1 minute, and a negative reaction is indicated by the reagent retaining its yellow colour (Cappuccino and Welsh 2020).

Methyl-Red Test

A loopful of the isolate under investigation was inoculated onto MR-VP broth and incubated at 37°C for 24-48 hours. Three drops of methyl red solution were added, and the colour change was observed. A colour change from light yellow to pink indicates a positive methyl red reaction (Chessbrough, 2020).

Voges-Proskauer Test

To the bottles containing MR-VP broth, using *a* wire loop, isolates were inoculated and incubated at 37°C for 24 hours. This was followed by the addition of 0.5ml of 6% alpha-naphthol solution and 0.5ml of 40% KOH, which were allowed to remain for 30 minutes. Development of pink colouration within 30 minutes indicates a positive reaction (Cheesbrough, 2020).

Citrate Utilization Test

Simmons' citrate agar slant was streaked back and forth with a light inoculum picked from the center of a well-isolated colony and incubated aerobically at 35 ^{0}C to 37 ^{0}C for up to 24-48 hours. Color change was observed from green to Prussian blue along the slant (Foebes *et al.*,2016)

Eosin Methylene Blue Agar Test

EMB agar plate was inoculated with the test organism and incubated at 35-37 ^{0}C for 18-24 hours. The plate was examined for colour, size and sheen (Cappuccino and Welsh, 2020)

Voges Proskauer test:

MR-VP broth was inoculated with the test organism and incubated at 35-37 ^{0}C for 24-48 hours.1 ml of broth was transferred to a clean tube, and 0.6ml of α-naphthol was added, followed by 0.2ml of 40% potassium hydroxide, with gentle shaking and exposure to air. The result was observed within 15-30 minutes for colour change from pink to red (Cheesbrough, 2020)

Oxidase test

Using a sterile inoculating wire loop, a small portion of the colony was transferred onto oxidase test paper impregnated with oxidase reagent and observed at room temperature. Color change was observed within 10-30 seconds (Cheesbrough, 2020).

Urease production

Urea agar slant was inoculated with the test organism and incubated at 35 ^{0}C to 37 ^{0}C .slant was examined at 4-6 hours for rapid urease producers up to 24 hours (Cheesbrough, 2020)

Pigment production test

Bacterial isolate was streaked onto King's A and B agar plates and incubated at 35 ^{0}C to 37 ^{0}C for 24-48 hours under aerobic conditions. Plates were examined for visible pigment. productionon the surface of colonies and diffusion of pigment into the surrounding medium. Greenish-blue (pyocyanin) on King's A, whileyellow-green fluorescent pigment was observed on King's B agar (Koneman *et al.*, 2021)

Test for Disinfection Sensitivities

Three commonly used disinfectants for toilet floor disinfection in the College of Natural and Pharmaceutical Sciences, Bayero University, Kano, were used in the present study.

X = Dettol (165ml) Active compound: Chloroxylenol, Isopropyl Alcohol; Excipients: Sapo Vegitalis, Oleum Pini, Aromaticum, Aqua, etc

Y = Hypo (500) Active compound: Sodium Hypochlorite; Excipients: Citric Soda, De-mineralized

Z = Royal Gad (150) Active compound: 32%Phenol; Excipients: Sapo Vegitalis

Inoculum Standardisation

The standard was prepared from the stock cultures maintained on nutrient agar slants and subcultured onto nutrient broth using a sterilized wire loop. The density of the suspension was determined by comparison with a 0.5 McFarland standard of Barium sulphate solution (Cheesbrough, 2017).

Screening for antibacterial activity of Disinfectant using the agar well diffusion method

For antibacterial testing, each isolate was tested in three independent biological replicates, and each biological replicate was assayed in three technical replicates. Each biological replicate was initiated from an isolated colony into 5-10ml Mueller-Hinton broth and incubated at 35-37 °C for 18-24 h to obtain fresh cultures. The agar well diffusion method, as described by Celestina (2020), was used to assess the antibacterial activity of the disinfectant. Three different concentrations were used (100%, 75%, and 50%). A suspension of the test organism previously adjusted to 0.5 McFarland standard (10^8CFU/ml) was inoculated onto the surface of sterile Mueller-Hinton agar plate. Three wells, each 9mm in diameter, were aseptically bored using a sterile cork borer on each agar plate. On each agar plate, an aliquot of 0.3 ml of the varying concentration was added to each well. This was done for all three replicates. The same procedure was applied for all the disinfectants. The plates were allowed to stand at room temperature for 15minutes to enable proper diffusion of the disinfectants. The plates were then incubated at 35°C for 18-24 hours. The effect of the disinfectant was assessed by measuring the diameters of the zones of inhibition to the nearest millilitre and comparing them with the standard (CLSI, 2023).

To determine the final working concentration, the disinfectants were prepared at 0.5%, 0.75%, and 1.0%, corresponding to 5000 μg/mL, 7500 μg/mL, and 10,000 μg/mL, respectively. For the control groups, sterile distilled water used for the dilution of test disinfectants served as the negative control, while a standard disinfectant served as the positive control.

Table A: Zone of inhibition interpretative table (CLSI, 2023)

Antibiotic (μg)	Susceptible	Intermediate	Resistant
Gentamicin10	≥21	16-20	≤15
Augmentin30	≥15	13-14	≤12
Ciprofloxacin10	≥20	17-19	≤16
Co-trimoxazole30	≥16	11-15	≤10
Streptomycin30	≥15	12-14	≤11
Ampicillin30	≥29	----	≤28
Cephalexin10	≥18	15-17	≤14
Ofloxacin10	≥16	13-15	≤12
Nalidixic acid30	≥20	15-19	≤14
Perfloxacin10	≥19	14-18	≤13

Antibiotic Susceptibility Testing Using Standard Antibiotics

The susceptibility of the isolates to some standard antibiotics was determined using the Kirby-Bauer disc diffusion method (Sharma, 2022) as shown in Table A. A sterile swab was dipped into the previously adjusted bacterial suspension to a 0.5 McFarland standard and used to inoculate the surface of a Mueller-Hinton agar plate, which was then allowed to stand for 5 minutes. Antibiotic disks were applied with sterile forceps, and the agar plates were incubated at 37°C for 24 hours. The zone of inhibition was measured and then compared with the standard (CLSI, 2023). The following antibiotics were used: Gentamicin 10 μg (CN), Augmentin 30 μg (AU), Ciprofloxacin 10 μg (CPX), Co-trimoxazole 30 μg (SXT), Streptomycin 30 μg (S), Ampicillin 30 μg (PN), Cephalexin 10 μg (CEP), Ofloxacin 10 μg (OFX), (NA) Nalidixic acid 30 μg, (PEF) Perfloxacin10μg.

Determination of Minimum Inhibitory Concentration

Determination of MIC, which is the lowest concentration of a specific antimicrobial or disinfectant needed to inhibit the growth of a known test organism. It was determined using the broth dilution method according to CLSI guidelines (CLSI, 2023). Disinfectant was prepared at concentrations of 100%, 75%, 50%, 25%, 12.5%, and 6.25%. The tubes containing equal volumes (1 ml) of Mueller-Hinton broth inoculated with standardized test organisms were then filled with 1 ml of the disinfectant concentrations. The tube containing only Muller-Hinton broth and bacteria without disinfectant serves as a negative control, while the positive control tube contains disinfectant and broth without bacteria. The tubes were then incubated for 18–24 hours, after which the amount of visible growth (turbidity) was observed. The procedure was repeated for all the disinfectants under test (Cheesbrough, 2020).

Determination of Minimum Bactericidal Concentration

A loop of inoculum from the MIC tubes that showed no growth was subcultured onto fresh Mueller-Hinton agar to determine the MBC (the lowest concentration of a specific antimicrobial or disinfectant that kills 99.9% of a given bacterium). The streaked Mueller-Hinton agar plates were incubated at 37°C for 24 hours and observed for growth. Mueller-Hinton agar plates showing no growth indicate a 99.9% bactericidal effect of the disinfectant at that concentration; this is the MBC (CLSI, 2023).

Statistical Analysis

Statistical analysis of the antibacterial activity of all three disinfectants was performed using SPSS 8.0. A unidirectional analysis of variance (ANOVA) approach was used, and the results obtained are presented as means ± SD. Post hoc tests were used to assess the prevalence of the isolates and to compare the disinfectant concentrations' activity at $p \leq 0.05$ (statistically significant).

RESULTS

Isolation and identification of bacterial isolates

Of 180 samples collected, five bacteria were isolated and identified based on morphological, cultural, and biochemical characteristics. These are: *Staphylococcus aureus, Escherichia coli, Klebsiella sp., Bacillus sp., Pseudomonas sp.* Total bacterial counts (Table 1) revealed that bacterial population density varied across different sampling areas (three faculties within the college). Colony counts of bacterial population from the faculties ranged from 3.38×10^5 cfu/ml to 7.3×10^6 cfu/ml. The prevalence of bacterial isolates (Table 2 and Figure 2) differed significantly among species. X^2=15.18, df =4. *S. aureus* had the highest prevalence (29.4%), and *Klebsiella sp* had the lowest prevalence (8.23%). Statistical distribution and prevalence of bacterial isolates recovered from toilet floors is presentend in Table 3. Table 4 shows the susceptibility of the disinfectants against the isolated bacteria. It was observed that at 100% concentration, all the disinfectants showed high activity against *S. aureus*, followed by *Bacillus sp.* Zones between 18mm and 32mm were observed.

Table 1: Total Bacterial Count (Density) across Faculties within the College

Faculty	Colony Count (cfu/ml)	Mean Bacterial Count (cfu/ml)	± SD
Life Sciences	4.56×10^6		
Physical Sciences	3.38×10^5	4.13×10^6	3.06×10^6
Pharmaceutical Sciences	7.50×10^6		

Table 2: Prevalence of Five Bacterial Isolates from Toilet Floors of Three Faculties within the College

Faculty	No of samples	Number of isolates (%prevalence)				
		A	B	C	D	E
Life Sciences	60	10(40.0)	09(39.1)	05(25.0)	05(50)	07(100)
Physical Sciences	60	07 (28.0)	08(34.8)	08(40.0)	00(00)	00(00)
Pharm. Sciences	60	08 (32.0)	06(26.1)	07(35.0)	05(50)	00(00)
Total	180	25(100)	23(100)	20(100)	10(100)	07(100)

KEY: A = *S.aureus*, B = *E.coli*, C = *Bacillus sp.*, D = *Pseudomonas sp.*, E = *Klebsiella sp.*

Table 3: Statistical Distribution and Prevalence of Bacterial Isolates Recovered from Toilet Floors

Isolates	Frequency	%Prevalence	95% Confidence	p-value
S.aureus	25	29.4	2	
E.coli	23	27.1	1	
Bacillus sp.	20	23.5	1	0.004
Pseudomonas sp.	10	11.2	6	
Klebsiella sp.	07	8.2	4	

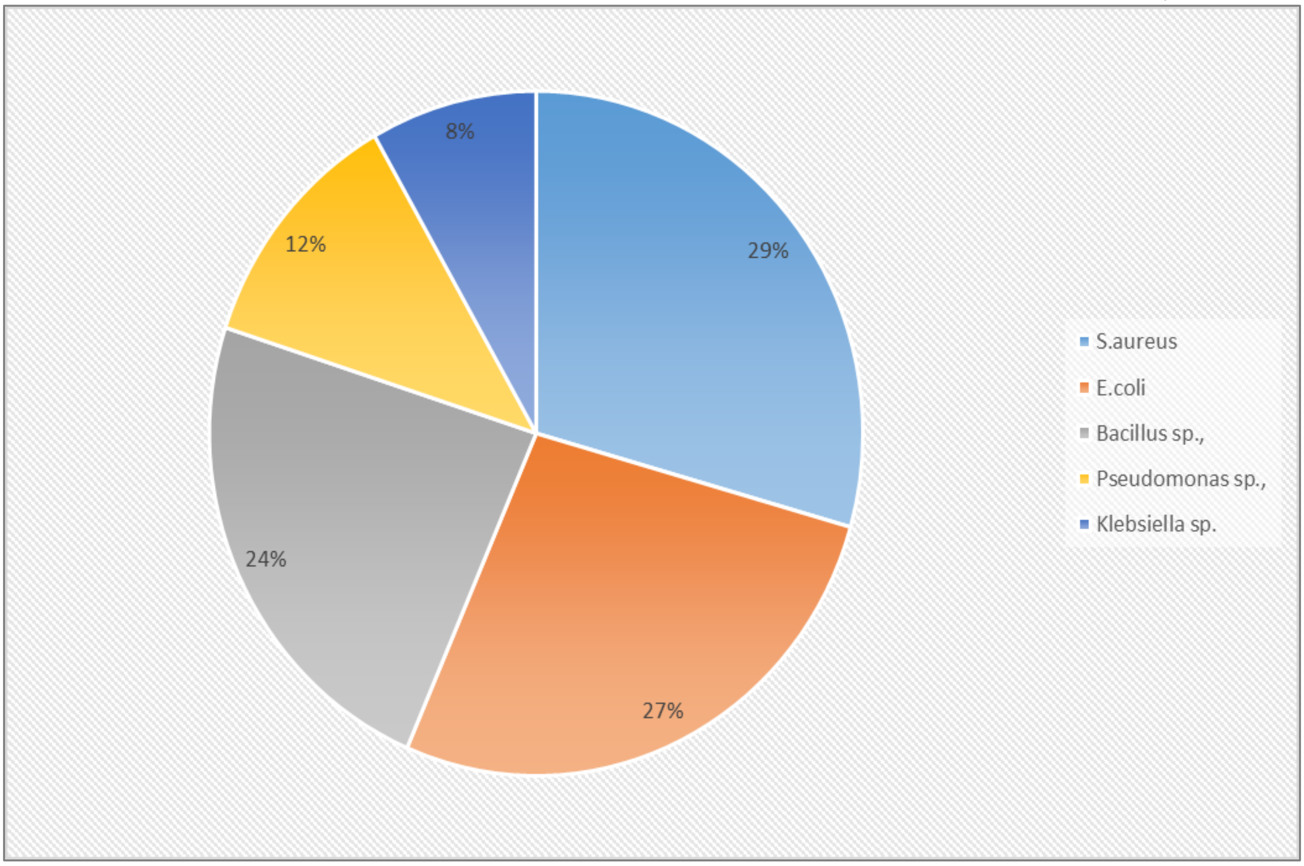

Figure 2: Percentage Prevalence of Bacterial Isolates

Table4: Summary of zones of inhibition of the three disinfectants against the isolates

TEST ORGANISM	CONCENTRATION (%), Zones (mm)								
	X			**Y**			**Z**		
	50	75	100	50	75	100	50	75	100
Escherichia coli	10	12	20	17	24	22	10	16	18
Bacillus sp	7	21	25	18	22	28	15	21	26
Staphylococcus aureus	13	18	20	22	27	32	18	20	30
Klebsiella sp	12	17	25	18	22	27	10	18	22
Pseudomonas sp	8	13	18	20	22	24	18	20	24

Table 5: Susceptibility Profile of bacterial isolates to antibiotics

Test organism	SUSCEPTIBITY PATTERN (mm)									
	CN	AU	CPX	SXT	S	PN	CEP	OFX	NA	PEF
E. coli	S	S	R	R	S	R	R	R	R	R
Bacillus sp.	S	S	S	S	R	I	S	S	S	S
S. aureus	S	S	S	S	S	R	R	S	S	S
Klebsiella sp.	S	S	S	S	R	R	R	R	R	S
Pseudomonas sp	S	S	S	R	S	I	S	S	S	S

Key: ER = Resistant, S = Susceptible, CN = Gentamycin (10cmg), AU = Augmentin (30cmg), CPX = Ciprofloxacin (10cmg), SXT = Co-trimoxazole (30cmg), S = Streptomycin (30cmg), PN = Ampicillin (30cmg), CEP = Cephalaxin (10cmg), OFX = Ofloxacin (10cmg), NA = Nalidixic acid (30cmg), PEF =Perfloxacin (10cmg)

Table 6: Minimum Inhibitory Concentration (MIC) /Minimum Bactericidal Concentration (MBC).

Test organism	MIC(mg/ml)			MBC (mg/ml)
	X	Y	Z	
S. aureus	6.25	6.25	6.25	6.25
E. coli	12.5	6.25	12.5	6.25
Klebsiella sp.	6.25	6.25	12.5	6.25
Pseudomonas sp	6.25	6.25	6.25	6.25
Bacillus sp.	6.25	6.25	6.25	6.25

Higher MIC values were observed for *E. coli* and *Klebsiella sp.* at 12.5 mg/ml, while S. aureus, *Bacillus sp.* And *Pseudomonas sp.* showed a lower MIC value of 6.25 mg/mL. All organisms exhibited uniform mbc values of 6.25, indicating comparable bactericidal activity across the isolates.

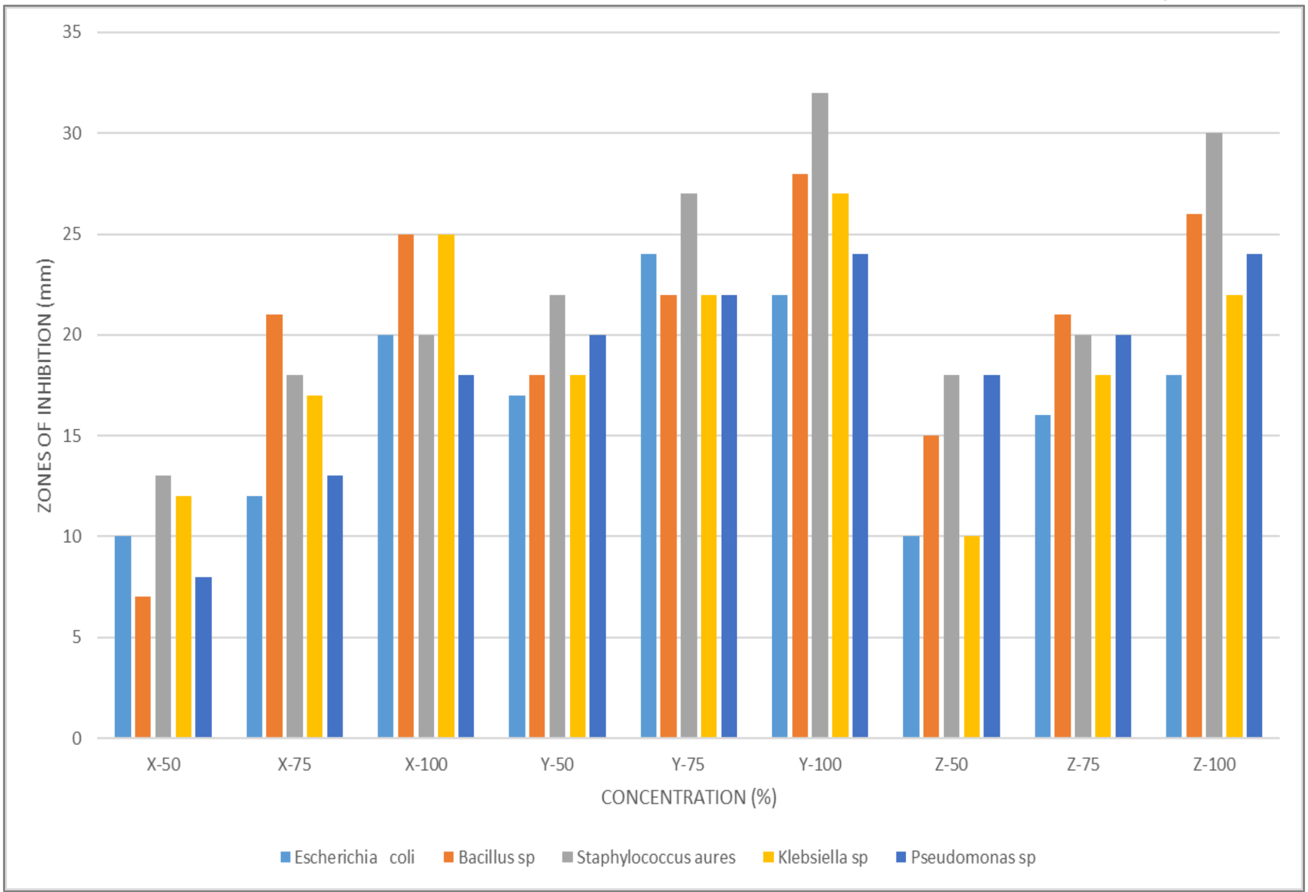

Figure 3: Zone of Inhibition (mm) of Disinfectant against the Isolated Bacteria

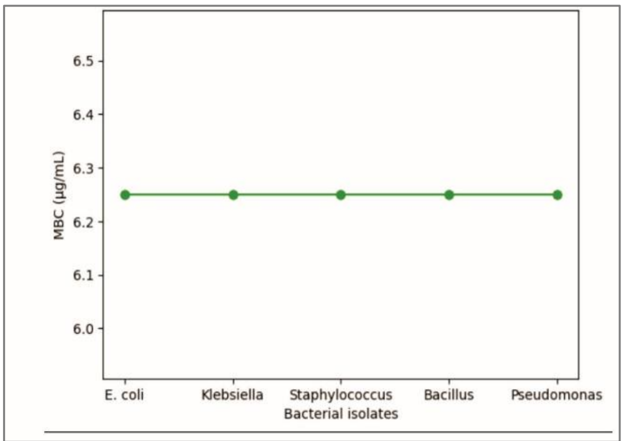

Figure 4: MBC Curve of Disinfectants Against Bacterial Isolates

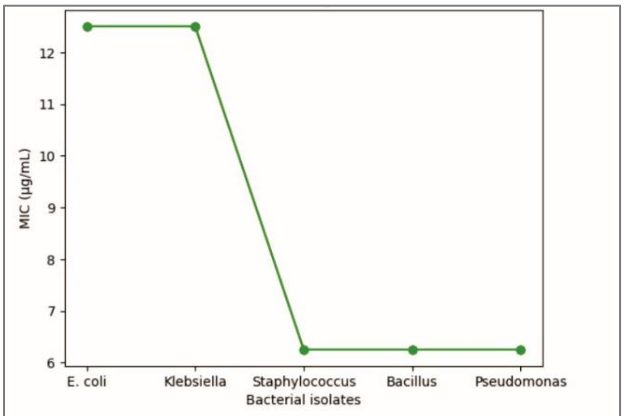

Figure 5: MIC Curve of Disinfectants Against Bacterial Isolates

Sensitivity Test Using Some Standard Antibiotics

The results of the antibiotic sensitivity test using some standard antibiotics are shown in Table 5 and Figure 3. Some of the isolates are resistant, yet still sensitive to the disinfectant used. All the isolates were found to be sensitive to three antibiotics: Augmentin, gentamicin, and ciprofloxacin. Zones were measured in mm, compared and interpreted using a standard.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

Results corresponding to the MIC were depicted in Table 6. The results showed that disinfectant X was effective *against S. aureus, Klebsiella sp and Bacillus sp.* It certainly inhibited their growth at 6.25mg/ml, while *E. coli* was inhibited at a concentration of 12.5mg/ml. The results showed variations in susceptibility among the bacterial species. The Minimum Bactericidal Concentration (MBC) for all disinfectants was 6.25mg/mL across all isolates. This characterizes the bactericidal effect of the disinfectants. MBC and MIC Curves of disinfectants against bacterial isolates are shown in Figure 4 and 5 respectively.

DISCUSSION

Disinfection of environmental surfaces, including toilet floors, in public institutions is essential. It remains the golden standard for the prevention and control of pathogen transmission and, consequently, for minimizing the risk of exposure to resistant strains (Chaoui et al., 2019). The present study assessed and compared the performance of some commonly used disinfectants against bacteria isolated from toilet floor surfaces, a place known to harbour diverse microbial contaminants (Artasensi *et al.,* 2021). The isolation of *S.aureus*, *E.coli*, *Bacillus sp*., *Pseudomonas sp*., and *Klebsiella sp.* aligns with previous studies reporting these organisms as common environmental and sanitation-related contaminants (Saccucci *et al*., 2018). The disinfectants demonstrated complete bactericidal activity at 100% concentration. This supports the manufacturer's recommendation, which specifies using disinfectants at the appropriate strength for effective sanitation. However, when diluted to 75% and especially 50%, its effectiveness decreases markedly. This concurs with previous reports that improper dilution reduces disinfectant potency and permits microbial survival, allowing pathogens to persist on toilet floors and increasing the risk of transmission, especially in public facilities (Suen *et al*., 2019). *Bacillus sp.* and *Pseudomonas sp.* showed the greatest tolerance. *Bacillus sp.* forms spores, enabling survival under harsh environmental conditions, including exposure to disinfectants. Pseudomonas sp. possesses intrinsic resistance mechanisms, such as efflux pumps and biofilm formation (Oluyege *et al*., 2015). Their survival under diluted conditions is therefore expected and poses serious hygiene implications. The comparative analysis revealed notable variations in the antibacterial effectiveness of the disinfectant with higher concentrations and broader-spectrum active ingredients demonstrating greater inhibitory effects against isolates. This agreed with previous studies, which reported that disinfectant efficacy is strongly influenced by concentration, contact time, dilution, and the intrinsic resistance of the target organisms (Artasensi *et al.,* 2021). *S. aureus* and *E. coli* were generally more susceptible to the disinfectants, showing lower minimum inhibitory concentrations. This may be attributed to the structural characteristics of these organisms. *S. aureus,* being Gram-positive, lacks an outer membrane, which may allow easier penetration by disinfectant agents. Similarly, although *E. coli* is Gram-negative, it is often susceptible to commonly used disinfectants when applied at recommended concentrations. The susceptibility of *E. coli* to the tested disinfectants implies that these disinfectants have the potential to control or eliminate this bacterial strain (Nwankwo and Offiah, 2016). In contrast, *Pseudomonas sp.* and *Klebsiella sp.* exhibited relatively higher resistance mechanisms, including low outer membrane permeability, efflux pumps, and biofilm formation, which reduce the effectiveness of many antimicrobial agents. The reduced susceptibility observed in this study aligns with the reports of Suen et al. (2019) and Sotohy et al. (2021), highlighting Pseudomonas as a persistent environmental contaminant capable of surviving harsh conditions and exposure to disinfectants. *Bacillus sp.* also showed a variable resistance pattern, which may be linked to its ability to form endospores. Endospores are highly resistant to chemical agents and environmental stress, making *Bacillus sp* more difficult to eliminate with routine disinfection, especially at lower concentrations (Davis *et al*., 2019). *S. aureus* and *E .coli* were generally more susceptible, although adaptive stress response can raise tolerance. The resistance pattern of the isolates to the tested antibiotic can affect the treatment of infectious diseases caused by them. Most of the isolates obtained in this study were resistant to streptomycin, ampicillin and cephalexin. Sokhn *et al.* (2020) reported the same resistant pattern in *E. coli* and *Klebsiella* and attributed it to the misuse and overuse of antibiotics. All the isolates are sensitive to gentamicin and augmentin, which concurs with the results of Munita and Arias (2016), who reported sensitivity as a result of their mechanism of action. All the disinfectants X (32% Phenol), Y (Sodium hypochlorite), and Z (Chloroxylenol, Isopropyl Alcohol) were active against all the organisms isolated, and their activity increased with increasing concentration (Ijeoma *et al*., 2016; Kamal *et al.,* 2019). Comparatively, the most effective disinfectant in this study demonstrated broad-spectrum activity against all tested isolates, while others showed selective or reduced activity. This highlights the need for informed selection of disinfectants based on their antimicrobial spectrum of activity rather than cost alone. Disinfectant Y (Sodium hypochlorite) has the highest potency against *S. aureus* and *E. coli*, and acts as the most broadly bactericidal agent in our panel, consistent with its rapid oxidative chemistry. Sodium hypochlorite generates free chlorine species that oxidize thiol groups, damage enzymes and membrane lipids, and cause irreversible protein inactivation and cell lysis (Artasensi *et al*., 2021). Phenolic formulations showed good activity against vegetative cells via membrane disruption, enzyme inhibition, and oxidative stress, but had slower action and limited sporicidal activity (Kapoor *et al*., 2017). The activity of disinfectant X was probably due to its mechanism of action. The poor activity of disinfectant Z observed in this study is likely due to the additional excipients used, which may include fragrance, emollients, humectants, and thickening agents. These could potentially prevent disinfectant from reaching the bacterial cells. The mechanisms of action of the disinfectants used play a role in the different activities observed in the organisms isolated (Rozman *et al*., 2021; Kapoor *et al*., 2017). The MIC and MBC values of all the disinfectants used in the study correspond to the manufacturers' instructions for dilution. The findings correspond to those of Matini et al. (2020), who found that the larger the zones of inhibition, the lower the Minimum Inhibitory Concentration (MIC).When the MIC is lower, it means that the disinfectant is more effective at inhibiting bacterial growth, leading to larger zones of inhibition (CLSI, 2023). Inhibition zones are a key measure of disinfectant and antibiotic effectiveness; their size reflects the disinfectant's ability to diffuse through the agar and inhibit bacteria. A larger zone indicates higher potency and efficiency of the disinfectant (Kowalska-krochmal, 2021). The concentration-dependent effectiveness observed in this study emphasizes the importance of using disinfectants at manufacturers recommended dilutions. Reduced efficacy at lower concentrations suggests that improper dilution practices, which are common in domestic and public settings, may contribute to the persistence of bacteria on toilet floors and increase the risk of disease transmission (Celestina, 2020). This is particularly important because a larger inhibition zone means that the disinfectant can work effectively at lower concentrations, benefiting both effectiveness and cost. Several studies and references support the correlation between inhibition zone size and antimicrobial efficacy, as well as the principles underlying inhibition and their relationship (CLSI, 2018; EUCAST, 2019). Several variables, including the type, concentration, contact time, test technique (in vitro and in vivo), target organism, and matrix, affect the activity of disinfectants (Kowalska-krochmal, 2021; Sabharwal, 2015). There is growing evidence that sub-lethal exposure and improper dilution/contact time select for disinfectant tolerance and co-select antibiotic resistance. Environmental and behavioural factors (organic load on floors, mixing errors, etc.) further reduce field effectiveness compared with the laboratory assay (Davies *et al.,* 2019). Generally, bacterial growth inhibition increased with increasing disinfectant concentration, indicating a concentration-dependent antibacterial effect. *S. aureus* and *Bacillus sp* were the most susceptible organisms, with growth inhibition observed at lower disinfectant concentration (50-75%). In contrast, *Pseudomonas sp* and *Klebsiella sp* exhibited higher resistance, requiring higher concentrations (75-100%) to achieve complete inhibition. *E. coli* shows moderate susceptibility, with the MIC value mainly observed at 75% concentration

CONCLUSION

Toilet floors harbor multiple bacterial species of public health concern. The disinfectants used in this study are highly effective at 100% concentration, eliminating all isolates. Disinfectant Y (sodium hypochlorite) has the largest zone of inhibition, "22mm-32mm". *Bacillus sp*. and Pseudomonas sp. exhibit the highest resistance, indicating potential challenges to sanitation if disinfectants are improperly diluted. This suggests the proper use of disinfectants at recommended concentrations for effective control of microbial contamination on toilet floors. The presence of antibiotic-resistant bacteria on the toilet floors in our institution is a public health concern.

RECOMMENDATIONS

- Disinfectants should always be used at full manufacturer's recommended concentration to ensure maximum microbial kill.
- Households, schools and public facilities should avoid excessive dilution of disinfectants during cleaning.
- Further studies should evaluate disinfectant performance against biofilms, as many toilet-associated bacteria can form them.
- Regular sanitation training should be provided for cleaners and facility staff.
- It is also recommended to regularly use other disinfectants at different concentrations.

REFERENCES

Abdulrahman, H., Noraidi, H. J., & Khalid. (2025). Practical guide to calculate sample size for chi-square test in biomedical research. *BMC Medical Research Methodology*, 25, 144. [Crossref]

Adegoke, A. A., & Komolafe, A. O. (2018). Antibacterial efficacy of household disinfectants against bacteria isolated from restroom facilities in Nigeria. *African Journal of Infectious Diseases*, 12(2), 54-61.

Akanbi, B. O., & Olurunfemi, B. O. (2020). Assessment of bacteriological contamination of public toilets surfaces and effectiveness of selected disinfectants in South-Western Nigeria. *African Journal of Clinical and Experimental Microbiology*, 213, 245-252.

Artasensi, A., Mazzotta, S., & Fumagalli, L. (2021). Back to basics: Choosing the appropriate surface disinfectant. *Antibiotics*, 10(6), Article 613. [Crossref]

Cappuccino, J. G., & Welsh, C. T. (2020). *Microbiology: A laboratory manual* (12th ed.). Pearson.

Celestina, M. I. (2020). Germicidal activities of some selected disinfectants on *S. aureus* and *E. coli*. *Journal of Microbiology & Biotechnology*, 5(2), Article 000161. [Crossref]

Centers for Disease Control and Prevention. (2016, December 9). *Chemical disinfectants*. [Link]

Chaoui, L., Mhand, R., Mellouki, F., & Rhallabi, R. (2019). Contamination of the surfaces of a health care environment by multidrug-resistant (MDR) bacteria. *International Journal of Microbiology*, 2019, Article 3236526. [Crossref]

Cheesbrough, M. (2017). *District laboratory manual practice in tropical countries* (3rd ed., Part 2). Cambridge University Press.

Cheesbrough, M. (2020). *District laboratory manual practice in tropical countries* (3rd ed., Part 2). Cambridge University Press.

Clinical and Laboratory Standards Institute. (2018). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically* (11th ed.). CLSI standard M07.

Clinical and Laboratory Standards Institute. (2023). *Methods for dilution antimicrobial susceptibility tests for* *bacteria that grow aerobically* (11th ed.). CLSI standard M07.

Davies, R., & Wales, A. J. (2019). Antimicrobial resistance on farms: A review including biosecurity and the potential role of disinfectants in resistance selection. *Comprehensive Reviews in Food Science and Food Safety*, 18(3), 753-774. [Crossref]

Elsernagy, M., Moussa, M., Ahsan, A., Khalfan, A., & Eissa, A. (2015). Exploratory study of bacterial contamination of different surfaces in four shopping malls in Sharja UAE. *Journal of Environmental and Occupational Science*, 4(1), 1-6. [Crossref]

European Committee on Antimicrobial Susceptibility Testing. (2019). *Breakpoint tables for interpretation of MICs and zone diameters* (Version 9.0). [Link]

Forbes, B., Sahm, D. F., & Weissfeld, A. S. (2017). *Bailey and Scott's diagnostic microbiology* (13th ed.). Elsevier.

Ijeoma, E., Ngozi, M., Egbuta, A., & Egberongbe, H. O. (2016). Characterization of microorganisms isolated from bathroom walls in a Nigerian University. *Journal of Applied Life Sciences International*, 9(4), 1-11. [Crossref]

Kamal, M. A., Khalaf, M. A., Ahmed, Z. A. M., & El Jakee, J. (2019). Evaluation of the efficacy of commonly used disinfectants against isolated chlorine-resistant strains from drinking water used in Egyptian cattle farms. *Veterinary World*, 12(12), 2025-2032. [Crossref]

Kapoor, G., Saigal, S., & Elongavan, A. (2017). Action and resistance mechanisms of antibiotics: A guide for clinicians. *Journal of Anaesthesiology Clinical Pharmacology*, 33(3), 300-305. [Crossref]

Khatoon, H., Chavan, D., Anokhe, A., & Kalia, V. (2022). Catalase test: A biochemical protocol for the bacterial identification. *AgriCos e-Newsletter,* 3(1), Article 18

Koneman, E. W., Procop, G. W., Church, D. L., Hall, G. S., & Janda, W. M. (2021). *Koneman's color atlas and textbook of diagnostic microbiology* (7th ed.). Wolters Kluwer.

Kowalska-Krochmal, B., & Dudek-Wicher, R. (2021). The minimum inhibitory concentration of antibiotics: Methods, interpretation, clinical relevance. *Pathogens*, 10(2), Article 165. [Crossref]

Matini, E., Shayeghi, F., Vaghar, M. E., Nematian, J., Hosseini, S. S., Mojri, N., Taherabadi, N. T., Hakimi, R., Ahmadi, N., Badkoubeh, N., Esmaeili, H., Akhlaghi, M., & Vaseghnia, H. (2020). A survey of public restrooms microbial contamination in Tehran city, capital of Iran, during 2019. *Journal of Family Medicine and Primary Care*, 9(6), 3131-3135. [Crossref]

Munita, J. M., & Arias, C. A. (2016). Mechanisms of antibiotic resistance. *Microbiology Spectrum*, 4(2). [Crossref]

Nwankwo, E. O., & Offia, L. C. (2016). Bacterial contamination of toilet surfaces in tertiary institutions and evaluation of commonly used disinfectants. *Nigerian Journal of Microbiology*, 30(1), 3285-3294.

Oluyege, A. O., [could not be automatically formatted - please provide full author names]. (2015). Microbial contaminants on frequently used surfaces in public areas and their sensitivity to disinfectants. *Journal of Applied Sciences and Environmental Management*, 19(4), 575-580.

Rozman, U., Pusnik, M., Kmetec, S., Duh, D., & Sostar Turk, S. (2021). Reduced susceptibility and increased resistance of bacteria against disinfectants: A systematic review. *Microorganisms*, 9(12), Article 2550. [Crossref]

Sabharwal, J. (2015). Health issues and environmental impact of cleaning agents. *International Journal of Novel Research in Life Sciences*, 2(2), 31-38.

Saccucci, M., Bruni, E., Uccelletti, D., et al. (2018). Surface disinfections: Present and future. *Journal of Nanomaterials*, 2018, Article 8950143. [Crossref]

Sharma, R. (2022, January 8). *Kirby Bauer disc diffusion method for antibiotic susceptibility testing*. Microbe Notes. [Link]

Sokhn, E. S., Salami, A., El Roz, A., Salloum, L., Bahmad, H. F., & Ghssein, G. (2020). Antimicrobial susceptibilities and laboratory profiles of *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* isolates as agents of urinary tract infection in Lebanon: Paving the way for better diagnostics. *Medical Sciences*, 8(3), Article 32. [Crossref]

Sotohy, A. S., Mobarez, A. A., & Diab, M. S. (2021). Evaluation of the efficiency of the most common commercial disinfectants against some pathogens isolated from New-valley poultry farms "in-vitro studies." *New Valley Veterinary Journal*, 1(1), 23-28. [Crossref]

Suen, L. K. P., Siu, G. H. K., Guo, Y. P., Yeung, S. K. W., & O'Donoghue, M. (2019). The public washroom - friend or foe? An observational study of washroom cleanliness combined with microbiological investigation of hand hygiene facilities. *Antimicrobial Resistance & Infection Control*, 8, Article 47. [Crossref]