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Assessment of the Microbiological Quality and Efficacy of Two Common Disinfectants Used in Hospital Laboratory

Enitan Seyi Samson[1, *], Ochei John Okeleke[1], Digban Kester Awharentomah[2], Akele Yomi Richard[3], Nwankwo Kasie Josephine[2], Arisi Chinedu Paul[1]

[1]Department of Medical Laboratory Science, Babcock University, Ilishan-Remo, Nigeria

[2]Department of Medical Laboratory Science, Igbinedion University, Okada, Nigeria

[3]Department of Medical Laboratory Science, Afe Babalola University, Ado-Ekiti, Nigeria

Email address

enitans@babcock.edu.ng (Enitan S. S.), ocheij@babcock.edu.ng (Ochei J. O.), kadigban@yahoo.com (Digban K. A.), akeler@abuad.edu.ng (Akele Y. R.), lilmisskasy@yahoo.co.uk (Nwankwo K. J.), arisiprosoer@gmail.com (Arisi C. P.)

[*]Corresponding author

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Abstract

The present study assessed the microbiological quality and efficacy of two common disinfectants (Jik and Lysol) used in a hospital laboratory. Sterility test using Nutrient Agar and Sabour Dextrose Agar plates incubated at 37°C and 25°C, respectively, were employed to detect the present of potential bacterial and fungal contaminants in 3 new batches of stock disinfectants. Swabs of work-bench surfaces designated as Site 1, 2 and 3 were collected in triplicate at the end of each business day (*i.e,* before disinfection) and also after disinfection with 30% Jik and 2.5% Lysol dilution and cultured in tubes containing 3 ml of Tryptic Soy Broth medium and 0.1 mL Neutralizer. Surface viable count was carried out to determine the bacterial population density of three sites pre-disinfection and post-disinfection. Colonies of bacteria were identified by Gram-stain, motility test and routine biochemical tests. The efficacy of the disinfectants against each bacterial isolate at 10 min contact time was determined using the quantitative suspension test. The killing rate of the disinfectants was expressed by plotting the logarithms of surviving cells (CFU/mL) against exposure time (min) of the disinfectant. The outcome of the study showed that the microbiological quality of the two disinfectants tested was satisfactory. Bacterial distribution pre-disinfection include: *Staphylococcus epidermidis*, *Enterococcus aerogenes, Proteus mirabilis, Bacillus subtilis, Pseudomonas aeruginosa* and *Klebsiellia pneumoniae*; while only *B. subtilis, P. aeruginosa* and *K. pneumoniae* were recovered post-disinfection. Lysol proved to be more potent than Jik at the dilution and contact time tested with a log reduction of bacterial population ≥5. *S. epidermidis*, *E. aerogenes* and *P. mirabilis* were completely killed by the two disinfectants within the 10 minutes contact time. While, a population density of 1-2 log CFU/ml of *B. subtilis, P. aeruginosa* and *K. pneumoniae* still survived after 10 min exposure to Lysol and Jik. The outcome of this study further strengthening earlier works and underscored the need to periodically assess the microbiological quality and efficacy of disinfectants routinely supplied to the laboratory to ensure proper control of infections by using right disinfectant in right concentration for a right contact time.

Keywords

Hospital Laboratory, Disinfection, Disinfectants, Jik, Lysol, Quality, Efficacy

1. Introduction

The hospital environments are fraught with bacteria and other microbes just waiting to infect staff working within the facility and patients who are often more susceptible to infections due to their illnesses. This is of serious concern, especially as the rate of multidrug-resistant organisms continues to rise and causing infections that are becoming increasingly more difficult to treat [1].

One of the cardinal principles of hospital care is that it should cause no harm to the patient. However, for many patients the outcome is different; they acquire infections in hospital. These are called "nosocomial infections", also known as "hospital acquired infections-*HAIs*". According to Ducel [2], nosocomial infection is an infection occurring in a patient in a hospital or other health care facility in whom there is no evidence that the infection was present or incubating at the time of hospital admission. This includes infection acquired in the hospital but appearing after discharge, and also occupational infections among staff of the facility [3].

Hospital acquired infections are a major problem throughout the world, associated with increased morbidity, high mortality and significant health care costs [4], [5]. Hospital environmental surfaces in contact with patients are infected by epidemiological important microorganisms which survive on different levels of the hospital [6].

Infection control is therefore, an important part of the culture in all hospitals. Rigorous cleaning methods are required to ensure that the hospital environments (*i.e,* laboratories, theaters, wards and equipment) are safe for the health workers and the patients [7].

The Medical Microbiology Laboratory in particular, is a special unit within the hospital environments. It is primarily saddled with the task of receiving clinical specimens such as urine, stool, CSF, blood, pus, vaginal, throat and wound swabs among several others in order to isolate and identify medically important bacterial pathogens from patients, carriers and environment up to the species level and also to perform antimicrobial susceptibility testing following internationally recognized methods. This daunting task is associated with high infection risk for the laboratory staff and the patients they serve, hence the need for proper comprehensive disinfection program in the laboratory.

Disinfection is a procedure that is use to reduce the level of microbial contamination on surfaces to the barest minimum. According to UNC [8], disinfection is generally a less lethal process than sterilization. It eliminates nearly all recognized pathogenic microorganisms but not necessarily all microbial forms (e.g., bacterial spores) on inanimate objects. Disinfection does not ensure "overkill" and therefore lacks the margin of safety achieved by sterilization procedures. In hospital practice, it is achieved either by surface cleaning or immersing the contaminated objects in a disinfectant [9].

Disinfectants are broad-spectrum biocidal compounds that inactivate microorganisms on inanimate surfaces [10]. Also, according to Division of Oral Health-Infection Control Glossary [11], disinfectants are antimicrobial agents that are applied to the surface of non-living objects to destroy microorganisms that are living on the objects. On one hand, effective use of disinfectants is an important factor in the prevention of nosocomial infections [12]; while on the other hand, improper use of disinfectants is an important factor as well in the spread of nosocomial infections [13].

Different types of commercial disinfectants with different chemical composition and trade names exist in the market today. However, major types of disinfectants include: Alcohols, Aldehydes, Oxidizing agents, Phenolics, Quaternary ammonium compounds, Silver, Copper alloy surfaces and Thymol-based disinfectants among several others [14].

According to Cheesbrough [15], no single disinfectant is likely to kill all micro-organisms in any sample of infected waste. Furthermore, all chemical agents of disinfection take time to act. The rate of disinfection is approximately logarithmic, with a plot of the logarithm of the number of survivors against unit time giving straight line over the most of the curve. This means that a constant proportion of surviving bacteria are killed per unit of time. The actual rate of kill varies due to certain factors, e.g., the nature of the disinfectant and concentration used. Environmental conditions also affect the efficiency of chemical disinfectants, e.g., the presence of organic matter, the pH of solution and the possible inactivation of chemical compounds such as soap [16].

Surface disinfectants are routinely used for decontamination of a variety of work areas in the hospitals including laboratories which minimizes the contamination of samples and media. Consequently, the disinfectants themselves have become a medium for microbial growth. It has been reported by researchers that sub-inhibitory level of disinfectants may induce sporulation and/or germination of *Clostridium deficile* spores [9]. Further it has been stated that contamination decreases the efficacy and effectiveness of disinfectants and multiple nosocomial outbreaks have taken place in the past as a result of ineffective disinfectants [16].

Activity against key pathogens and lethal speed are the most important factors in disinfectant choice [18]. The use of safe and effective disinfectant solution with minimal damage to equipment and personnel is one of the fundamental principles of disinfection. None of the disinfectants are suitable for all different needs; therefore choosing the most appropriate disinfectant at any given time is very critical in infection control programs in the hospital [13].

The disinfectant activities rely on the consistent quality and efficacy of the disinfectants, which is of great significance in ensuring the final result of infection control [6]. Hence, testing the quality and efficacy of disinfectants is very important component in hospital infection control, but largely overlooked by many hospitals [19].

While a consistent disinfection practice is cardinal to reducing hospital acquired infections, the need to ensure that

potent disinfectants are used to achieve efficient disinfection cannot be underscored. Due to the differences in the strains of clinical isolates, continuous emergence and re-emergence of antibiotic-resistant isolates, the choice of drugs and disinfectants use in hospital laboratory, as well as the disparity in the potency of different batches of disinfectants used in the same hospital laboratory resulting in differences in disinfectant susceptibilities even amongst the same strain of bacteria; there is therefore the need to evaluate and re-evaluate the microbiological quality and efficacy of disinfectants been used in hospital-laboratory from time to time.

Against this backdrop, the present study was design to evaluate the microbiological quality and efficacy of two common disinfectants use in the Microbiology Laboratory of the Babcock University Teaching Hospital (BUTH), Ilishan-Remo, Ogun State, Nigeria; with the specific objectives of establishing the presence of potential microbial contaminants in the disinfectants, determine the bacterial distribution and counts before and after the disinfection of the laboratory bench surfaces; as well as to evaluate the individual killing rate of the two disinfectants for each bacterial isolate.

2. Materials and Methods

2.1. Study Design

This was a prospective, observational and analytical study.

2.2. Study Area

The study was carried out at the Department of Medical Microbiology Laboratory of Babcock University Teaching Hospital (BUTH), Ilishan-Remo, Ogun State. BUTH is a 300-bed space capacity Private Hospital and the only Tertiary Medical Centre in the community. While, Ilishan-Remo community is one of the geopolitical wards in Ikenne Local Government Area of Ogun state, situated in the tropical area of South-western part of Nigeria, coordinates: 7°29′00″N 2°53′00″E.

2.3. Study Period

The study lasted for a period of two months (April-May, 2017).

2.4. Ethical Approval

Ethical approval for the study was sought for from the Babcock University Health Research Ethical Committee (BUHREC), while administrative approval was obtained from the Management of the Babcock University Teaching Hospital (BUTH), Ilishan-Remo, Ogun State.

2.5. Test Disinfectants, Disinfectant Control, Neutralizer and Diluent

Three (3) new batches of Royal Gad Lysol® (50% Cresol v/v) manufactured by AG Industries Limited, Ibadan, Oyo State, Nigeria, with NAFDAC Reg-No-A4-3924 and Jik® (3.5% M/V Sodium Hypochlorite solution) manufactured by Reckitt Benckiser, Limited, Agbara, Ogun State, Nigeria, with NAFDAC Reg-No-02-0356, were obtained from the store officer of the hospital. These two agents are commonly used in most hospital-environments, including the Department of Medical Microbiology Laboratory of BUTH, because they are less toxic, cheap and readily available. Sterile distilled was used as disinfectant control. The neutralizer consisted of a mixture of equal volumes of 1% Sodium thiosulphate and 0.1% Tween 80; while sterile saline was used as a diluent.

2.6. Sterility Check of Test Disinfectants

Before carrying out the sterility check as indicated below, the expiry date of the products was noted. Method described by Maurer [20] was used to detect the presence of bacterial and fungal contaminants in the disinfectants used. Briefly, a 0.1 ml sample of each disinfectant was added to 0.9 ml sterile diluents which also contain 0.1 ml neutralizer to neutralize the residual activity of the disinfectant. Ten drops, each of 0.02 mL volume of the diluted sample was placed on each nutrient agar (NA) and Sabour dextrose agar (SDA) plates prepared. The NA plate was incubated at 37°C for three days, while the SDA plate was incubated at room temperature (25°C) for seven days. Five or more colonies on either plate indicate contamination of the test disinfectant. Fungal isolates were identified on the basis of microscopic (using Lactophenol cotton blue staining) and macroscopic characteristics (with the aid of an Atlas of Mycology) as described by Rajesh and Rattan [21].

2.7. Sample Collection

Swab samples were collected before and after work-bench surface disinfection is carried out. Sites of sample collection were designated as Site 1, Site 2 and Site 3.

2.7.1. Before Disinfection

Sampling of the entire work-bench surfaces (Site 1, 2 and 3) in the Medical Microbiology Laboratory Department of BUTH was collected in triplicate at the end of each business day (*i.e,* before disinfection), using sterile cotton swabs moistened with sterile saline. The swabs were placed in screw-capped tubes containing 3 ml of tryptic soy broth (TSB) medium.

2.7.2. After Disinfection

Afterwards, the entire work-bench surfaces (Site 1, 2 and 3) was disinfected by each of the test disinfectant according to the manufacturer’s recommended concentration for surfaces disinfection and after the stipulated contact time and drying of the disinfectant; sampling of the work-bench surfaces was repeated again as described earlier and cultured in tubes containing 3 ml of tryptic soy broth (TSB) medium and 0.1 mL neutralizer.

2.8. Sample Culture

For culturing the samples, the screw-capped tubes

containing each sample was manually shaken vigorously for proper mixing and care taken to avoid spillage. Then 0.002 mL of the sample was inoculated unto plates containing Blood agar (BA) medium and MacConkey agar (MCA) medium using a sterile calibrated wire loop. This was incubated at 37°C for 18-24 hours as described by Mokhtari *et al.* [22].

2.9. Determination of Population Density Pre- and Post-Disinfection

Surface viable count as described by Miles and Misra [23], was carried out to determine the surface microbial count before and after disinfection. Briefly, using ten-fold dilution, 1ml each of the broth culture from each site in the laboratory was diluted in 9 mls of sterile normal saline. The organisms were diluted serially up to 10^{10}. Each inoculum was plated out on nutrient agar as drops from a calibrated dropping pipette. Each drop was 0.02ml in volume and approximately 50 drops of such gave a volume size of 1 ml. Each drop was made to fall from a height of 2.5 cm onto the medium, where it spreads over an area of 1 cm in diameter.

For all isolates, 0.02ml of 10^4 to 10^9 dilutions was dropped in triplicates, *i.e* each of three plates received one drop of each dilution in separate numbered sectors. These plates were incubated for 24 hrs. After incubation, different numbers of colonies was obtained at different dilutions for the different test isolates. Counts were made in the drop areas showing the largest number of colonies without confluence; the mean of the three counts gives the viable count per 0.02ml of the dilution. Hence, the population density (CFU/ml) was calculated using the formula:

Mean no of colonies x no of drops/ml x dilution factor

The surface reduction rate of the disinfectant was calculated using the following formula:

log10 reduction = log10 pre-disinfection count - log10 post-disinfection count.

2.10. Isolation of Pure Cultures

Pure cultures of isolate within mixed bacterial population were obtained using the streak plate technique as described by Ochei and Kolhatkar [24]. Aseptic streaking of the inoculum with the aid a wire loop resulted in continuous dilution of the innoculum to give well separated surface colonies.

2.11. Identification of Bacterial Isolates

After incubation, plates containing cultured samples were investigated (before and after disinfection). Colonies of bacteria were identified by Gram-stain, motility test and routine biochemical tests such as determining the fermentation of glucose, lactose and sucrose in the triple sugar iron medium, urea hydrolysis, producing indole from tryptophan, use of citrate, producing hydrogen sulfide, determining the method of fermentation in methyl red Voges-Proskauer, oxidase production, catalase and coagulase production as described by Cheesbrough [25] and Mokhtari *et al.* [22]. The results of the above tests were entered into IDENTAX bacterial identifier (a free software developed using Sun Microsystems's Java Technology) for the taxonomical identification of bacteria isolates using phenotypical characteristics.

2.12. Evaluation of Disinfectant Activity on Each Test Isolate

Evaluation of the efficacy of the disinfectants against each microbial isolate was determined using the quantitative suspension test as described by Merap *et al.* [26] and USEPA [27].

2.12.1. Standardization of Organism

A single isolated colony of bacteria was removed from tryptic soy agar (TSA) plates and grown separately in 10 ml of tryptic soy broth (TSB) for 24 hour at 37°C. After incubation, the 24-hour broth culture was filtered with a saline pre-wet filter paper in order to remove slime and centrifuged for 20 minutes at 2000 rpm with a rotor centrifuge.

Afterwards, the cell pellets were washed with 10 ml of TSB. Then the population density of the bacterial suspensions in the TSB (about 10^7 CFU/mL) was adjusted to match that of 0.5 McFarland Standard (10^5 CFU/ml) by making a dilution of 1:100 in sterile TSB.

2.12.2. Quantitative Suspension Test (QST)

In brief, 0.1mL of the standardized bacterial suspension will be added to 0.9 mL of the disinfectant solutions and mix gently at room temperature for contact times of 0, 1, 3, 5 and 10 minutes. The timer was started when the test bacterial suspension and disinfectant are combined. Then at Time X, the specified contact time, 0.1 mL of the disinfectant-organism mixture was removed and transferred to a tube containing 0.9 mL of neutralizer (the 10^0 designated as Tube A) and mix thoroughly. Within 5 minutes of the transfer to the neutralizer tube, three additional ten-fold dilutions in saline blanks shall be made to achieve 10^{-1}, 10^{-2} and 10^{-3} dilutions (designated Tube B, Tube C and Tube D, respectively).

0.1 mL of each dilution was placed onto TSA plates in duplicate by the spread-plate technique and incubated at 37°C for 24 hours. After incubation, the TSA plates were observed for any visible growth. The surviving microbial colonies was enumerated, multiplied with factor hundred (100) and expressed as colony forming unit per milliliter (CFU/mL).

Controls were put up for all the test organisms to show the activity of the neutralizer. For control, 0.1 mL each of 0.5 McFarland broth of the test organism was mixed with 0.9 mL of neutralizer in separate tubes then transferred to TSB, as the procedure described with disinfectants. Later all the controls was streaked onto TSA plates. Presence of growth indicates that the neutralizer is not inhibiting the microbes tested.

Similarly 0.1 mL of each disinfectant was mixed with 0.9mL of neutralizer, then 0.1 mL suspension of the test

organism (0.5 McFarland standard) was added to each tube, later directly transferred and incubated in TSB and streaked on TSA plates. Growth on TSA plates shows effective neutralization of the disinfectant activity [27].

2.12.3. Determination of Bactericidal Effect of the Disinfectants

The bactericidal effect (Logarithm reduction factor) of the disinfectants was determined by subtracting the logarithm of the survivors after disinfectant contact from the logarithm of the original inoculum in control plates, using the following formula:

$$\text{Logarithmic Reduction Factor (RF)} = \text{Log } Nc - \text{Log } Nd$$

Where:

Nc = Number of colonies from control plates (No disinfectant)

Nd = Number of colonies from test plates (after contact with disinfectant)

Log10 reductions of 5 or more were taken as an indication of satisfactory microbicidal activity, *i.e*, at least 99.99% of the organisms killed.

2.12.4. Determination of the Killing Rate of the Disinfectants

The killing rate of the disinfectants on the other hand, was expressed by plotting the logarithms of surviving cells (CFU/mL) against exposure time (min) of the disinfectant as described by Kelsey and Maurer [28].

2.13. Data Analyses

Data was presented using tables. Statistical analysis was carried out with Paired-Samples T-Test using SPSS Statistics Software Package (Version 18.0) to test for significant differences between the efficacies of the two disinfectants to be tested. P-values <0.05 was considered significant [29].

3. Results and Discussion

The present study assessed the microbiological quality and efficacy of two common disinfectants (Jik and Lysol) used in the indoor environment of the Medical Microbiology Laboratory Department of Babcock University Teaching Hospital (BUTH), Ilishan-Remo, Ogun State, Nigeria. Sterility test was carried out on new batches of Jik and Lysol supplied. The microbiological quality of the two disinfectants tested is presented in Table 1. None of the 3 batches of Lysol tested had bacterial or fungal growth after appropriate days of incubation on Nutrient Agar (NA) and Sabour Dextrose Agar (SDA), respectively. Similarly, the 3 batches of Jik tested were free from bacterial growth; however, 1 or 2 colonies of either Microsporum spp, Trichophyton spp or Aspergillus spp were recovered from the Jik. But, since the number of fungal colonies recovered were less than 5, the microbiological quality of the disinfectants was considered satisfactory for use.

The mean bacterial counts before and after disinfection with 30% Jik is presented in Table 2. Bacterial population density on bench surfaces were reduced as follows: Bench 1: 12 to 9 Log CFU/ml, Bench 2: 11 to 8 Log CFU/ml and Bench 3: 12 to 9 Log CFU/ml, with a log reduction of 3, 3 and 2, respectively. Although the mean bacterial counts on the benches were significantly reduced after disinfection with Jik at P= 0.015, the bactericidal activity of the disinfectant at the dilution tested was considered not satisfactory since the Log reduction obtained was less than 5.

Table 1. *Microbiological quality of Lysol and Jik.*

Disinfectant	Batch Number	Media	Growth	No. of bacterial/fungal colonies counted	Isolate
Lysol	1	NA	-	0	None
		SDA	-	0	None
	2	NA	-	0	None
		SDA	-	0	None
	3	NA	-	0	None
		SDA	-	0	None
Jik	1	NA	+	-	None
		SDA	-	2	Microsporum spp, Trichophyton spp
	2	NA	-	0	None
		SDA	-	1	Aspergillus spp
	3	NA	-	0	None
		SDA	+	2	Microsporum spp

NB: ≥5 colonies indicate contamination of the test disinfectant.

KEY: NA = Nutrient Agar, SDA = Sabour Dextrose Agar, - = Absent, + = Present

Table 2. *Mean bacterial counts before and after disinfection with Jik.*

Bench Number	Mean Bacterial count before disinfection (Log CFU/ml)	Mean Bacterial count after disinfection (Log CFU/ml)	Log reduction (Log CFU/ml)
1	12.00	9.00	3.00*
2	11.00	8.00	3.00*
3	12.00	10.00	2.00*

*Although the mean bacterial count was significantly reduced after disinfection with Jik at P= 0.015 (P<0.05 is considered statistically significant), the bactericidal activity of the disinfectant at the dilution tested was considered not satisfactory since Log reduction was <5.

Also, the mean bacterial counts before and after disinfection with 2.5% Lysol solution is presented in Table 3. Bacterial population density on bench surfaces were reduced as follows: Bench 1: 12 to 5 Log CFU/ml, Bench 2: 9 to 4 Log CFU/ml and Bench 3: 11 to 5 Log CFU/ml, with a log reduction of 7, 5 and 6, respectively. The mean bacterial count was significantly reduced after disinfection with Lysol at *P= 0.01*. The bactericidal activity of the disinfectant at the dilution tested was considered satisfactory since Log reduction obtained was greater than or equal to 5.

The mean±SEM log reduction of bacterial population density by 30% Jik and 2.5% Lysol solution after ten minutes contact time was 2.67±0.33 Log CFU/ml and 6.00±0.58 Log CFU/ml, respectively (Figure 1). The Log reduction by Lysol was significantly higher than that of Jik at *P-value = 0.038*. Lysol showed a more satisfactory bactericidal activity than Jik at the dilution tested since its Log reduction was ≥5.

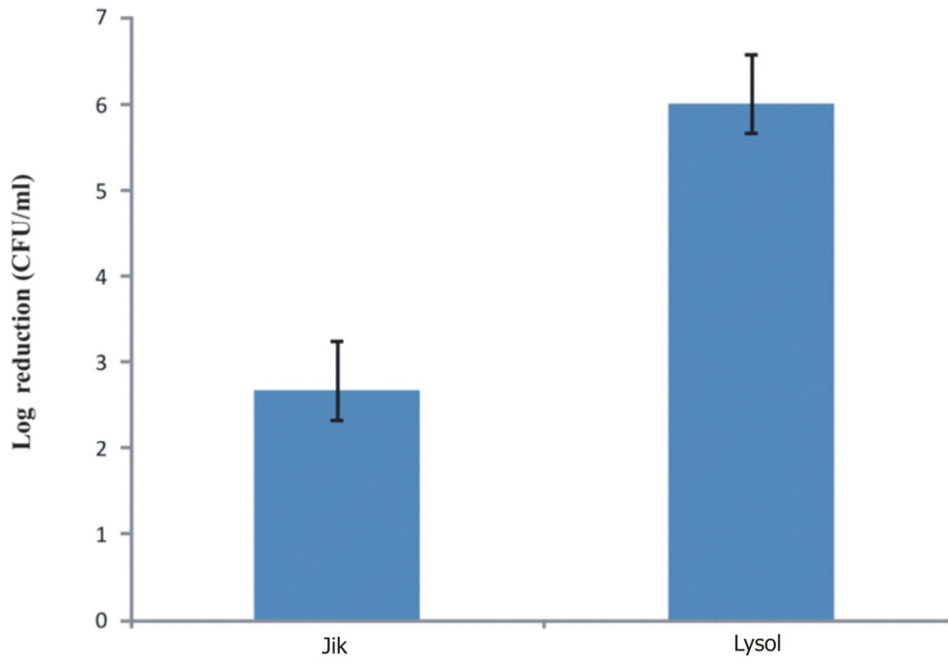


Figure 1. *A bar chart showing the mean±SEM log reduction of bacterial population density by Jik and Lysol after ten minutes contact time.*

Table 3. *Mean bacterial counts before and after disinfection with Lysol.*

Bench Number	Mean Bacterial Count before disinfection (Log CFU/ml)	Mean Bacterial Count after disinfection (Log CFU/ml)	Log reduction (Log CFU/ml)
1	12.00	5.00	7.00
2	9.00	4.00	5.00
3	11.00	5.00	6.00

*Mean bacterial count was significantly reduced after disinfection with Lysol at P= 0.01 (P<0.05 is considered statistically significant). The bactericidal activity of the disinfectant at the dilution tested was considered satisfactory since Log reduction was ≥5.

The distribution of bacteria isolates before and after disinfection with 30% Jik is presented in Table 4. Bacterial pathogens isolated from bench surfaces pre-disinfection include: *Bacillus subtilis, Staphylococcus epidemidis, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*; however, following disinfection, only *B. subtilis, P. aeruginosa* and *K. pneumoniae* were recovered from bench 1, 2 and 3, respectively.

Table 4. *Distribution of bacteria isolates before and after disinfection with Jik.*

Isolates	Before disinfection			After disinfection		
	Bench 1	Bench 2	Bench 3	Bench 1	Bench 2	Bench 3
Bacillus subtilis	+	-	-	+	-	-
Staphylococcus epidemidis	+	+	+	-	-	-
Pseudomonas aeruginosa	-	+	-	-	+	-
Klebsiella pneumoniae	-	-	+	-	-	+
No. of isolates per bench	2	2	2	1	1	1

Similarly, the distribution of bacterial isolates before and after disinfection with 2.5% Lysol solution is presented in Table 5. Bacterial pathogens isolated from bench surfaces pre-disinfection include: *Bacillus subtilis, Enterobacter aerogenes, Staphylococcus epidemidis,* and *Proteus mirabilis*; however, following disinfection, only *B. subtilis*

was recovered from bench 1 and none from bench 2 and 3.

Furthermore, bactericidal effect of 30% Jik and 2.5% Lysol solution on isolates per contact time is presented in Table 6 and 7, respectively. On one hand, growth was recorded for all the isolates exposed to 30% Jik, except for *E. aerogenes, S. epidemidis* and *P. mirabilis* at 10 minutes contact time. But on the other hand, 5 minutes contact time was sufficient for the 99.9% killing of the same, except for *P. mirabilis* that was killed at 10 minutes contact when 2.5% Lysol was used. Also, it was also observed that 10 minutes contact time was not sufficient for the killing of *B. subtilis, K. pneumoniae* and *P. aeruginosa*, as evident by growth on agar plates.

The killing rate of 30% Jik and 2.5% Lysol dilution for each bacterial isolate is presented in Figure 2 and 3, respectively, using line charts. The log of surviving cells remained virtually constant for the control (*i.e*, organism + neutralizer only) throughout the 10 minutes contact time; whereas for the test (*i.e,* organism + disinfectant + neutralizer), it varies with different contact time. There was no log reduction of *B. subtilis* in the first minute. But a log reduction of 2 was observed in each case in the 5th min. A surviving bacterial population of 2 Log CFU/ml and 1 Log CFU/ml, respectively, was observed at the end of 10 minutes contact time with Jik and Lysol (Figure 2a and 3a).

Table 5. *Distribution of bacterial isolates before and after disinfection with Lysol.*

Isolates	Before disinfection			After disinfection		
	Bench 1	Bench 2	Bench 3	Bench 1	Bench 2	Bench 3
Bacillus subtilis	-	+	-	-	+	-
Enterobacter aerogenes	+	-	+	-	-	-
Staphylococcus epidemidis	+	+	+	-	-	-
Proteus mirabilis	-	+	+	-	-	-
No. of isolates per bench	2	3	3	0	1	0

Table 6. *Bactericidal effect of Jik on isolates per contact time.*

Isolates	Contact Time				
	0 minute	1 minute	3 minutes	5 minutes	10 minutes
B. subtilis	G	G	G	G	G
E. aerogenes	G	G	G	G	NG*
S. epidemidis	G	G	G	G	NG*
P. mirabilis	G	G	G	G	NG*
K. pneumoniae	G	G	G	G	G
p. aeruginosa	G	G	G	G	G

Key: G = Growth, NG = No growth, * 10 minutes contact time was sufficient for the killing of the isolate.

Table 7. *Bactericidal effect of Lysol on isolates per contact time.*

Isolates	Contact Time				
	0 minute	1 minute	3 minutes	5 minutes	10 minutes
B. subtilis	G	G	G	G	G
E. aerogenes	G	G	G	NG*	NG
S. epidemidis	G	G	G	NG*	NG
P. mirabilis	G	G	G	G	NG
K. pneumoniae	G	G	G	G	G
P. aeruginosa	G	G	G	G	G

Key: G = Growth, NG = No growth, *5 minutes contact time was sufficient for the killing of the isolate.

Still, there was a log reduction of 1 and 2 for *E. aerogenes* in the first minute of exposure to 30% Jik and 2.5% Lysol dilution, respectively. Howbeit at the 5th min, 30% Jik dilution gave a log reduction of 3 for *E. aerogenes*, while a zero bacterial population was recorded for 2.5% Lysol dilution (Figure 2b and 3b).

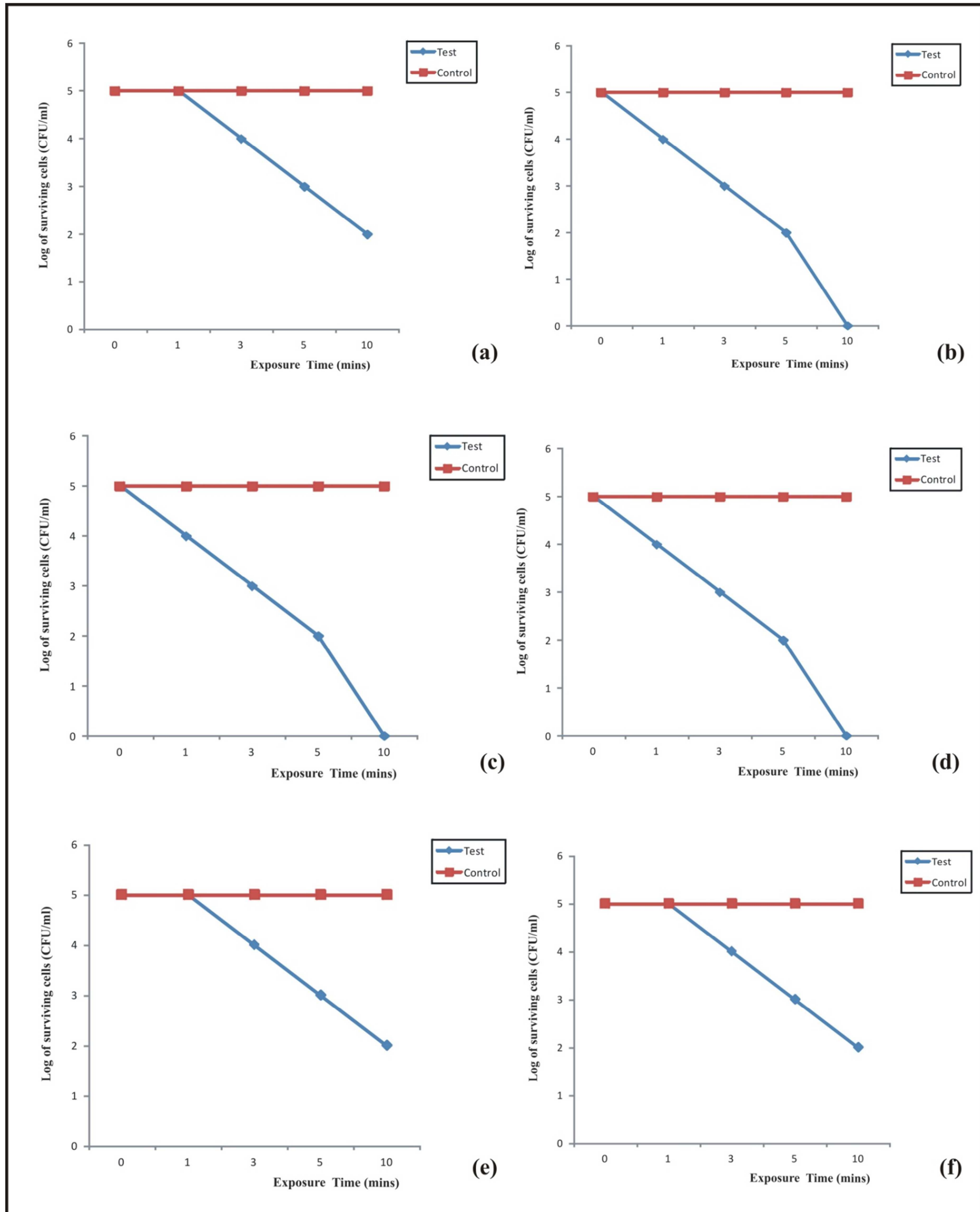
For *S. epidermidis*, 30% Jik and 2.5% Lysol dilution gave a log reduction of 1 in the first minute, but a log reduction of 2 and 3, respectively, at the 3rd min. And while bacterial population was reduced to 2 Log CFU/Ml at the 5th min by Jik, it had dropped to zero for Lysol (Figure 2c and 3c).

Furthermore, both 30% Jik and 2.5% Lysol dilution gave a log reduction of 1, 2, 3 and 5, respectively in the 1st, 3rd, 5th and 10th min of contact with *P. mirabilis* (Figure 2d and 3d). On the other hand, no Log reduction of *K. pneumoniae* was observed when exposed to 30% Jik dilution in the first min; howbeit, there was log reduction of 1 when exposed to 2.5% Lysol dilution. By the 5th min, both disinfectants gave a log reduction of 2 and 3, respectively. At the 10th min contact time, a surviving bacterial population of 2 Log CFU/ml and 1 Log CFU/ml, respectively, was observed for Jik and Lysol (Figure 2e and 3e). Also, there was no log reduction of *P. aeruginosa* in the first minute of contact with Jik, but there was Log reduction of 1 for Lysol.

While log reduction of 1 was observed for Jik at the 3rd min, it was 2 for Lysol. At the 5th min contact time, log reduction was 2 and 3, respectively. Howbeit, at the end of 10 minutes contact time, a surviving bacterial population of 2 Log CFU/ml and 1 Log CFU/ml, respectively, was observed after exposure to Jik and Lysol (Figure 2f and 3f). The

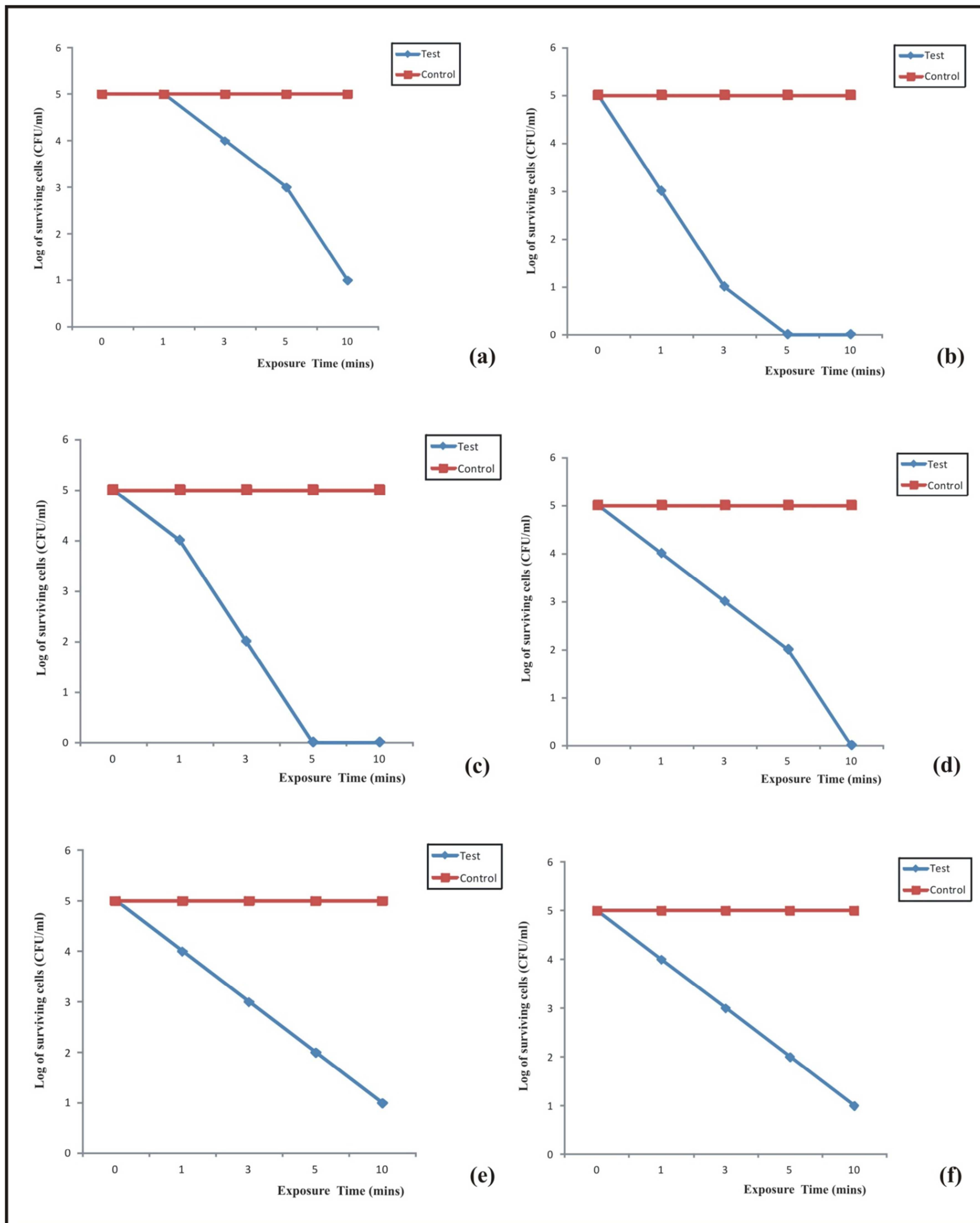
phenotypical characteristics of the bacterial pathogens isolated from the culture of bench surface swab are presented in Table 8.

The present study assess the microbiological quality and efficacy of two common disinfectants (Lysol and Jik) used in the indoor environments of the Medical Microbiology Laboratory Department of Babcock University Teaching Hospital, Ilishan-Remo, Ogun State, Nigeria. The microbiological assessment of 3 batches of disinfectants tested show that they all possess satisfactory microbiological quality, as no single bacterial colony was recovered from the disinfectants. Although 1 or 2 colonies of either Microsporum spp, Trichophyton spp or Aspergillus spp were particularly recovered from the 3 batches of Jik examined. But, since the number of colonies counted, were less than 5, the disinfectants were considered as "Not contaminated".



Key: a = B. subtilis, b = E. aerogenes, c = S. epidermidis, d = P. mirabilis, e = K. pneumoniae, f = P. aeruginosa

Figure 2. *Line charts showing killing rate of bacterial isolates when exposed to a 30% Jik dilution for 10 minutes.*



Key: a = B. subtilis, b = E. aerogenes, c = S. epidermidis, d = P. mirabilis, e = K. pneumoniae, f = P. aeruginosa

Figure 3. *Line charts showing killing rate of bacterial isolates when exposed to 2.5% Lysol dilution for 10 minutes.*

Table 8. *Phenotypical characteristics of the bacterial pathogens isolated from culture of bench surface swabs.*

Isolate	GR	Shape	Mot	Ure	Oxi	Ind	Cit	VP	MR	Cat	Coa	Glu	Suc	Lac
B. subtilis	+	Bacilli	+	-	-	-	+	+	-	+	-	+	+	-
E. aerogenes	-	Bacilli	+	-	-	-	+	+	-	-	-	+	+	+
S. epidermidis	+	Cocci	-	+	-	-	-	-	-	+	-	+	+	+
P. mirabilis	-	Bacilli	+	+	-	-	+	-	+	+	-	+	-	-
K. pneumoniae	-	Bacilli	-	+	-	-	+	+	-	-	-	+	+	+
P. aeruginosa	-	Bacilli	+	+	+	-	+	-	-	+	-	-	-	-

Key: GR = Gram reaction, Mot = Motility test, Urea = Urease test, Ox = Oxidase test, Ind = Indole test, Cit = Citrate test, VP = Voges-Proskauer, MR = Methyl red test, Cat = Catalase test, Coa = Coagulase test, Glu = Glucose, Suc = Sucrose, Lac = Lactose

The outcome of this work differs from those of Oie and Kamiya [30] and Zembrzuska [31], who reported a total microbial plate count of *Pseudomonas aeruginosa* ranging from <10 CFU/mL to 3.62 X 10^4 CFU/mL and from 10^2 to 10^8 CFU/mL of *P. aeruginosa*, *P. fluorescens* and others, respectively, from two new disinfectants tested. It also differ from the work of Gajadhar *et al.* [17], who reported that 11 (6.1%) out of the 180 samples of disinfectant examined in four major hospitals in Trinidad, were heavily contaminated by Pseudomonas spp.

The zero bacterial count recorded for the two test disinfectants (Lysol and Jik), as well as the lack of significant number of fungal colonies recovered from the Jik only, show that the 3 batches of the two disinfectants tested are both safe for laboratory use without any risk of infection, although the possibility of contamination during their usage exist.

With regard to the efficacy of the test disinfectants, a higher Log reduction of bacterial load was achieved with Lysol than with Jik. The bactericidal activity of 30% Jik dilution was considered not microbiologically satisfactory since the Log reduction obtained was less than 5. But on the other hand, the bactericidal activity of 2.5% Lysol dilution was considered microbiologically satisfactory since the Log reduction obtained was greater than or equal to 5.

With regard to the distribution of bacteria isolates; *Bacillus subtilis, Staphylococcus epidermidis, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were present on bench surfaces before disinfection; however, following 10 minutes contact time of disinfection with 30% Jik dilution, only *B. subtilis, P. aeruginosa* and *K. pneumoniae* were recovered from the bench surfaces. On the other hand, *Bacillus subtilis, Enterobacter aerogenes, Staphylococcus epidermidis* and *Proteus mirabilis* were present pre-disinfection; but following disinfection with 2.5% Lysol dilution, only *B. subtilis* was recovered from one of the benches.

The current study is similar to the work done by Obi *et al.* [32], who isolated arrays of bacteria from the operating theatre of Usmanu Danfodiyo University Teaching Hospital Sokoto, Sokoto State. The outcome of their work show that *S. epidermidis, B. subtilis* and others were sensitive to Izal and Povidone Iodine at the different concentrations tested.

The recovery of bacterial isolates post-disinfection in this present work agrees with work of Yi-Shan *et al.* [33], who recovered colonies of *Staphylococcus aureus* from some sampling swabs following initial disinfection of mechanical ventilator and face plates using 0.5% Hypochlorite.

This confirms the existence of inadequate disinfection practices in our hospitals which may be due to low potency, inadequate dilution and contact time of the disinfectant used or it could be due to the inherent resistance of the bacterial isolates to the test disinfectants. For instance, Radcliffe *et al.* [34] reported that two strains of *Enterococcus faecalis* were significantly resistant to hypochlorite than the other species tested. They however noted that, as the concentration of hypochlorite was increased, the time taken to reduce numbers of CFU below the limit of detection decreased. After 30-min contact time, even 0.5% hypochlorite had reduced viable counts below the limit of detection. And when higher concentrations were employed, less time was required. They found out that 5.25% hypochlorite was completely effective by 2.0 min.

No doubt, antibiotic resistant strains exist and some of which have also shown cross resistance to some disinfectants. It must be stressed here, that bacterial cells that remained viable after disinfection of the bench surfaces are obviously resistant to the disinfectants, howbeit; the mechanism by which bacteria acquire resistance to disinfectants is not well understood and required further investigation.

However, the possible protective effect of organic matter upon the bacterial cells during disinfection must be considered. In an experiments performed by Virto *et al.* [35], with a calculated organic load of 1,120 ppm, the concentration of hypochlorite (10%) had to be raised several times to achieve bacterial inactivation. The disinfectant dose had a clear but differential effect on the bacterial strains only above 15–35 mg/L, which was in contrast to the low chlorine concentration (approximately 1 mg/L) necessary to completely inactivate the same microbial populations when tested in a distilled water milieu.

Furthermore, except for *B. subtilis, K. pneumoniae* and *P. aeruginosa* with a surviving bacterial population of 1-2 Log CFU/ml, all the test isolates were completely killed within 10 min when exposed to Jik and Lysol. The outcome of this present study agrees with the work of Bipasa *et al*. [36], who observed that at a concentration of 2.5% (1:40 dilution) and 10 min contact time, lysol was unable to kill *P. aeruginosa* and *K. pneumoniae*, but the same were killed when concentration was increased to 10% (1:10 dilution) within 30 seconds. Their work also showed that a concentration as high as 20% (1:5 dilution) which is 4 times the recommended concentration and 2 hours contact time was required to effectively killed spores of *B. subtilis.*

This study also agrees with the work of Wijesinghe and Weerasinghe [37], who reported a failure with Lysol to achieve a log reduction of 5 against *P. aeruginosa* at the manufacture's recommended use dilution and a contact time of 15 minutes. They however, observed a significant bactericidal effect on the said organism at a concentration twice higher than the recommended use dilution of Lysol.

But, on the other hand, this study differ from the work of Prasanthi *et al*. [19], who observed no surviving bacterial population of *P. aeruginosa* and *B. subtilis* when exposed to 1% hypochlorite for 5 min contact. It also differs from the work of Awodele *et al*. [38], who observed a mean zone of inhibition of 4 and 15 mm for *B. Substilis* and *P. aeruginosa*, respectively when tested against 50% Jik dilution. Still, the current study also partly disagrees with the work of Rutala *et al.* [39] who reported approximately log reduction of 4 and > 5.5 of *P. aeruginosa* when exposed to Lysol Disinfectant and Lysol Antibacterial Kitchen, respectively for 5 min. The

disparity observed in the result of this present study when compared to those of previous studies is not unconnected to the inherent potency and dilution of disinfectants used.

With regard to the role of a neutralizer in the assessment of the microbicidal effectiveness of disinfectants; a neutralizer is an agent which posses the ability to quench the action of a disinfectant or an antiseptic. As matter of fact, an effective neutralizer must satisfy the following criteria. First, the neutralizer must effectively inhibit the action of the biocidal solution. Second, the neutralizer must not itself be unduly toxic to the challenge organisms. Finally, the neutralizer and active agent must not combine to form a toxic compound. It is important to mention here that, the neutralizer utilized in this study did not have any growth inhibition on the test organisms. This observation agrees with work of Awodele *et al*. [38].

Sources of bacterial contamination of the bench surfaces in the Medical Microbiology Laboratory appear to be diverse and traceable to a variety of factors. These include the Staff's own normal flora, laboratory coats, foot wares, bags, gloves and hands, activity of Staff like sneezing, coughing, talking and yawning, frequent movement in and out of the laboratory, spillage of patient's clinical specimens while being processed and as well as outdoor air entering the laboratory. House-keeping activity such as sweeping or using dry dust mops can aerosolize particles that may contain microorganisms which may eventually settled on the bench surfaces. All these contribute to the bacterial load found in this research work before disinfection of the bench surfaces was carried out.

In addition; the organisms isolated and tested in this study are known to be common contaminants and colonizers of patients, intensive care units, operation theatres and laboratory surfaces. Their presence poses a great risk from public health protection perspective. *Enterobacter aerogenes* for instance, though a part of normal flora of the human gut, is an opportunistic pathogen. When normal host defenses are inadequate—particularly in infancy or old age, in the terminal stages of other diseases, after immunosuppression, or with indwelling venous or urethral catheters— localized clinically important infections can result, and the bacteria may reach the bloodstream and cause sepsis. It has been implicated in a broad range of hospital-acquired infections (such as pneumonia, urinary tract infections and wound infections) and occasionally causes community-acquired infections. The bacteria become pathogenic only when they reach tissues outside of their normal intestinal or other less common normal microbiota sites. Most strains possess a chromosomal β-lactamase called *ampC*, which renders them intrinsically resistant to ampicillin and first- and second-generation cephalosporins. Mutants may hyperproduce β-lactamase, conferring resistance to third generation cephalosporins [40].

S. epidermidis are usually carried on the skin of many healthy people and have long been referred to as apathogenic, but their role as pathogens and their increasing incidence have been recognized and studied in recent years, important infections due to coagulase-negative staphylococcus include central nervous system shunt infections, urinary tract infections and endophthalmitis [41].

P. mirabilis on the other hand are naturally found in the intestinal tract, but have been isolated from urine and pus, in addition to fecal specimen. It has been implicated in nosocomial infections, including: Urinary tract infection, septicemia, abdominal and wound infection. Also it is a secondary invader of ulcer, burn, pressure sores and chronic discharging ear. As an opportunist, it is frequently resistant to antibiotics [42], [43].

Bacillus subtilis are saprophytic organisms prevalent in soil, water, and air and on vegetation and are capable of causing disease in immune compromised humans [44]. This micro-organism is able to grow at temperatures higher than 32°C as given in the human body but it is known as usually non-pathogenic. In some cases, *B. subtilis* was isolated from surgical wound or tumor drainages, but it remained locally restricted and did not influence the course of wound healing. Incidents of progressive dissipating bacterial infections caused by *B. subtilis* (among other species) were only reported for highly immuno-deficient patients suffering e.g. from leukemia [45].

P. aeruginosa can be found in the intestinal tract, water, soil and sewage and is frequently found in moist environments in hospitals. Many infections with *P. aeruginosa* are opportunistic hospital-acquired, affecting those with low immune status. Infections are often difficult to eradicate due to development of resistance to antimicrobials. The pathogen is incriminated in skin infections (wounds, ulcers and sores), urinary tract infections, respiratory infections and external ear infections [41].

Meanwhile, *K. pneumoniae* is present in the respiratory tract and feces of about 5% of normal individuals. It causes causes chest infections and occasionally severe bronchopneumonia with lung abscesses. *K pneumoniae* can produce extensive hemorrhagic necrotizing consolidation of the lung. It can also cause urinary tract infection and bacteremia with focal lesions in debilitated patients. It ranks among the top ten bacterial pathogens responsible for hospital-acquired infections. It is second only to *E. coli* as a urinary tract pathogen [40], [41], [46].

4. Conclusion

The microbiological quality of the two disinfectants tested was considered satisfactory, as no significant number of microbial contaminants were recovered from them following sterility test. With regard to the bactericidal activity of the disinfectants, Lysol proved to be more potent than Jik at the dilution and contact time tested, since it gave a log reduction of bacterial population greater than or equal to 5. The bactericidal activity of the two disinfectants however, was time-dependent. The outcome of this study show that there is a need to periodically assess the microbiological quality and efficacy of disinfectants routinely supplied to the laboratory or hospital to ensure proper control of infections by using

right disinfectant in right concentration for a right contact time. We therefore recommend that, future Researchers should attempt to investigate the effect of temperature, higher concentration and prolonged contact time on the efficacy of these disinfectants.

Competing Interests

Authors have declared that no competing interests exist.

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