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Establishment of biocidal activity evaluation study protocol in healthcare facility for routine monitoring of antibacterial power of disinfectants

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Abstract

Mitigation of the nosocomial infection risk is of prime importance in any healthcare facility to protect patients as well as the working staff's health. To ensure accomplishing this target, one of the crucial good practices is the establishment of rigorous sanitization and disinfection programs that should be executed as part of GxP activity in hospitals. The cornerstone of this task is the implementation of a biocidal validation protocol to evaluate the commercially available antimicrobial formulae in the market against the microorganisms that could be found in the environment. In the present study, a biochemically identified – at least to the genus level -Gram-negative microorganism was found on three different occasions belonging to the *Burkholderia cepacia* complex (BCC). The disinfectant validation program was executed using four chemically different antimicrobial formulae that embraced alcohols, amphoteric detergent and peroxygen products. A preliminary neutralization study design evaluation was established before the biocidal assessment to ensure effective stoppage of the antimicrobial activity after a specific contact time with the microorganism using a bacterial count range between 40 and 100 Colony Forming Unit (CFU) which was determined by serial dilution. An acceptable neutralization (in terms of toxicity and efficacy) was achieved through a combination of chemical and dilution methods. Contact surface method for testing the biocides was applied using three different coupons that were made for the material of construction of the covers or lining of the walls, floors and metallic surfaces. Microbial reduction level exceeded 15,800 times the original count of $\geq 1 \times 10^6$ CFU/coupon with all groups and replicates at zero- and two-weeks storage time of the prepared and diluted disinfecting solution. The disinfectants that have been challenged in this study showed acceptable activity against the vegetative organism of concern in the healthcare industry with 2-hydroxypropane being slightly the least active among the group.

Keywords: disinfection, neutralizer, efficacy, toxicity, peroxygen, alcohol

1. Introduction

Nosocomial infections are a major problem of concern in healthcare buildings, especially in hospital facilities (1). They can be caused by various microorganisms, notably bacteria. Cross-contamination might also occur due to the transfer of the infectious agents between different sections of the plant and between departments through various means, including personnel, tools, instruments, furniture and equipment (2). Individuals with variable health defects might be at great risk from not only pathogenic microorganisms but also opportunistic and even commensal microbes (3). Depending on the state of illness, the exposure to these microorganisms may encounter health complications and even death depending on different factors such as route of entry, inoculum size, exposure length presence or absence of natural body barriers (4).

One of the major strategies in limiting the transmission and spreading of microorganisms through surfaces is the implementation of an effective sanitization program in the hospitals and other healthcare organizations using a validated disinfection protocol as a part of the GxP program for minimizing contamination and infection risk in healthcare facilities (5, 6). Several experimental designs have been adopted to test the antimicrobial power of biocidal products (7). It is important for the test protocol to be able to demonstrate an acceptable kinetic of the microbial death by the biocidal formula using a high microbial count to ensure the ability to control the bioburden level in a short time when high workload and traffic are expected (8 – 10).

The present study aimed to study the biocidal effect of four selected commercial disinfectant formulae from market retail in a healthcare setting. These disinfectants were used to test the antimicrobial effect on simulated surfaces that are common and could be found in the building. The study would be projected as a routine activity that should be implemented using simple tools and instruments in the laboratory as an integral part of GxP in hospitals and other healthcare facilities.

2. Materials and Methods

Biocidal validation protocol was established as a part of GxP activity that was aimed to be used in healthcare organizations,

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especially hospitals which will be implemented through standard procedures after an acceptable evaluation and verification program (11, 12). The test group in this work will be referred to as treatment synonymously as well as column in electronic dataset generation.

2.1. Selection of the biocidal agents

Disinfectant products were purchased from a market retail involving four types comprising three main classes of the biocidal agents: two alcohols (ethyl hydroxide and dimethyl carbinol) amphoteric detergent (Ampholyte with pH ≈ 8)), and Peroxygen compounds (acetyl hydroperoxide/perhydroxic acid in acidic formula) (13 - 15). A detailed description of very close products could be found in other sanitization study series for preparation and dilution.

2.2. Identification of the study subject

Establishment of an environmental microorganism library database was established electronically through dynamic inclusion of the biochemically identified microorganisms after appropriate Gram-stain implementation for microscopical examination. The identification was conducted using suitable miniaturized identification kit system such as API® and VITEK® 2 BioMerieux (16, 17). Upon the validation study execution, randomly selected microbial samples were used based on the health risk encountered with the critically ill patients, specifically for those in the Intensive Care Units (ICUs).

2.3. Preliminary neutralization study design

Three distinct neutralization groupings should be established as comparison treatments to evaluate the neutralization process. General procedure and steps were performed as outlined by researchers in other studies (18 – 20).

2.3.1. Viability group

This treatment involved only the microorganism without a chemical neutralizer alone or biocidal compounds. This group was prepared and serially diluted in sterile saline or buffer to deliver microbial plate count between 30 and 100 Colony Forming Unit (CFU) per nutrient agar plate. This treatment was used as a reference control to assess the possible toxicity on the microorganism that could be stemmed from the neutralizer components.

2.3.2. Neutralizer toxicity group

This treatment was composed of the microorganism plus the chemical neutralizer as a diluent. This group served as toxicity test against the viability group but represented the control for the evaluation of the efficacy of the neutralization procedure in the presence of the biocidal test materials. There should not be any adverse effect from the neutralizer components on the microbial viability which would be indicated by the relative recovery from that of the concurrent control treatment. The selected neutralizer was prepared and sterilized as described in a previous research study.

2.3.3. Neutralizer efficacy group

This treatment included the working concentration of the disinfectant that was diluted and mixed well with the neutralizer and adding the microorganisms. This group is the test treatment that was assessed against the toxicity group as a control. Thus, four lines of analysis were streamed based on the number of disinfectants to be evaluated. Judgment on a successful neutralization procedure would be based on the relative recovery as in the toxicity analysis.

2.4. Disinfectant validation experiment (13, 15)

Antimicrobial efficacy of the freshly prepared disinfectants was evaluated after dilution as recommended by the manufacturer to 70% (v/v) for alcohols, 2% (v/v) for peroxygens and 1% (v/v) for the ampholyte with purified water. The biocidal agents were added over representative coupon surface materials that were inoculated with the test microorganisms at ≥ 1,000,000 CFU/coupon which was determined using a serial dilution of the microorganisms in saline or buffer in tubes. The surfaces were made from representative materials for the construction of walls, floors and metallic surfaces (tools, instruments and furniture such as tables, and chairs). Antimicrobial Activity of the stored disinfectant over the study period (biocidal stability) after 14 days was evaluated using the same experimental procedures and conditions with the same microorganisms on the same surfaces.

2.5. Statistical evaluation of the study (21)

2.5.1. Neutralization design evaluation

This computation analysis should be performed to assess the validity and the quality of the neutralization design to avoid any unintentional bias in the results due to exaggerated effect from the residual amounts of antimicrobial component(s) in the medium (22).

2.5.1.1. Neutralizer toxicity and efficacy acceptance criterion

The accepted microbial recovery percent of the test from the reference control should not be less than 75%. Any result that could not meet the acceptance criterion must be assessed statistically to elucidate the significance before judging the outcome as true failure of the neutralization (22). The statistical significance of the difference between the reference values of the control values and the experiment groups was tested at significance level of 0.05. Statistical evaluation between the recovery ratio of the treatment groups against reference target values was also assessed using nonparametric analogue of t-test.

2.5.1.2. Recovery ratio and groups comparison

Recovery ratios of the experimental groups were compared using Analysis of Variance (ANOVA) at $\alpha = 0.05$. When the result is significant, a pairwise multiple comparison test was executed to elucidate the source of variation (22). When no evidence of significance could be found, the second line of comparison was performed on the separate treatments (as CFU count per plate) to determine the test group that shows a significant difference from that of control at $P = 0.05$. Cases

must be evaluated individually to decide to either proceed with the next step or halt the study of the concerned biocidal agent till further investigation.

2.5.2 Biocidal activity evaluation

The microbial count decline over the preselected contact time (i.e., five minutes in the current work) should not be less than 1000 times from the initial bioburden (23). A conservative threshold was set to 10,000 times decline in the number of the CFUs. The observed results would be recorded in Excel sheet database and any non-conforming input cells would be highlighted to spot the aberrant values. Unless there is no microorganism could be detected, the variation in log count of the recovered bioburden from exposure to disinfectant at zero- and two-weeks storage period should be within 0.3 – 0.5 (23).

3. Results

It would be plausible to start the analysis of the output results by visualizing data by describing the set of the results followed by a more comprehensive study of the neutralization design followed by the disinfectant evaluation.

3.1. Descriptive statistical interpretation of results of the treatments

The mean microbial recovery of Amphoteric Detergent, Ethyl Hydroxide, Dimethyl Carbinol and Acetyl hydroperoxide/Perhydroxic acid (abbreviated as TX, AD, EH, DC and AP, respectively) were 0.8820, 0.8243, 0.8637, 0.8257 and 0.8813, respectively whereas the standard deviations 0.03830, 0.02055, 0.007572, 0.02237 and 0.01002 in the same order. On the other hand, the calculated standard error of the mean (SEM) was 0.02211, 0.01186, 0.004372, 0.01291 and 0.005783. The lower 95% Confidence Interval (CI) of the (mean), (median) and (geometric mean) was (0.7869, 0.7733, 0.8449, 0.7701, 0.8565), (0.8490, 0.8030, 0.8550, 0.8000, 0.8700) and (0.7919, 0.7746, 0.8450, 0.7713, 0.8567). The computed upper 95% CI of (mean), (median) and (Geometric Mean (GM)) – in the same order – was (0.9771, 0.8754, 0.8825, 0.8812, 0.9062), (0.9240, 0.8440, 0.8690, 0.8410, 0.8890) and (0.9812, 0.8769, 0.8827, 0.8834, 0.9066). On the same line, the calculated (actual median), (discrepancy) and (GM) was (0.8730, 0.8260, 0.8670, 0.8360, 0.8850), (-0.1230, -0.07600, -0.1170, -0.08600, -0.1350) and (0.8815, 0.8242, 0.8636, 0.8255, 0.8813), respectively. Fig. 1 and 2 showed relative recoveries expressed as GM with CI and medians with Interquartile Ranges (IQR), respectively.

3.1. Recovery of tests groups vs. control count and the reference criterion

The plate count results of the test groups were not significantly different from that of the control of either neutralizer toxicity or biocidal neutralization efficiency which was indicated by the exact P-value of 0.25. For paired neutralizer validation experimental design, a two-sided (two-tailed) Wilcoxon matched-pairs signed-rank test was implemented at a statistical significance of $P < 0.05$ for raw data. The sum of positive and negative ranks was 0 and -6, respectively. The sum of the signed ranks (W) was -6. The median of the differences between control and test for toxicity and efficacy groups of AD, EH, DC and AP were -8, -12, -8, -10 and -7, respectively. The pairing was perfect with a significant correlation at $P > 0.05$ and the value of P (one-tailed) was 0.1667.

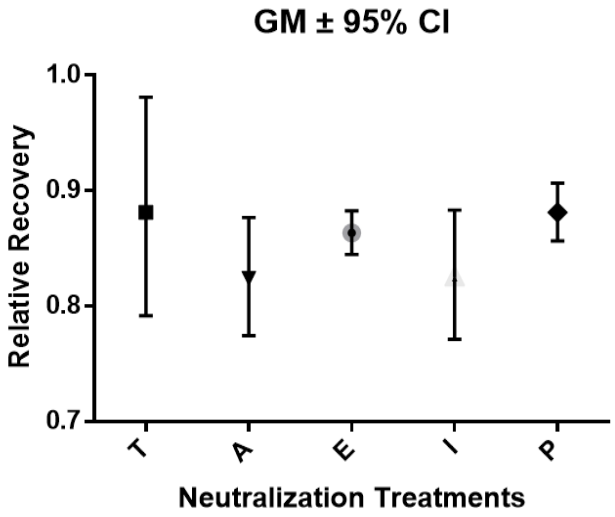

Fig. 1. Geometric Mean (GM) with 95% Confidence Interval (CI) for the relative recoveries of the neutralizer toxicity (T) and efficacy treatments of Amphoteric detergent, Ethyl hydroxide, Dimethyl carbinol and Acetyl hydroperoxide/perhydroxic acid (abbreviated as T, A, E, D and A, respectively)

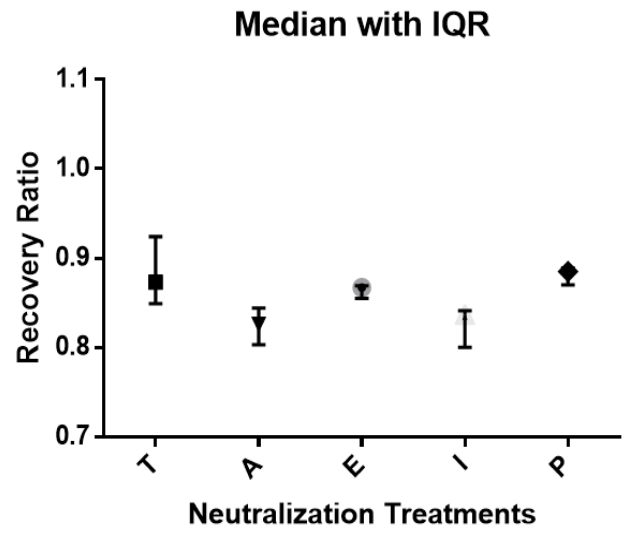

Fig. 2. Medians with Interquartile Ranges (IQR) for the relative recoveries of the neutralizer toxicity (T) and efficacy treatments of Amphoteric detergent, Ethyl hydroxide, Dimethyl carbinol and Acetyl hydroperoxide/perhydroxic acid (abbreviated as T, A, E, D and A, respectively)

The result of all treatment groups had met the acceptance criterion of 75% at $\alpha = 0.05$ with 95% confidence of the true mean to be within 0.75 and 1.00 recovery ratio. The microbiological count recovery for each group is shown in Fig. 3 on a logarithmic scale (at the y-axis) with the Error percent of the mean plate count expressed as CFU.

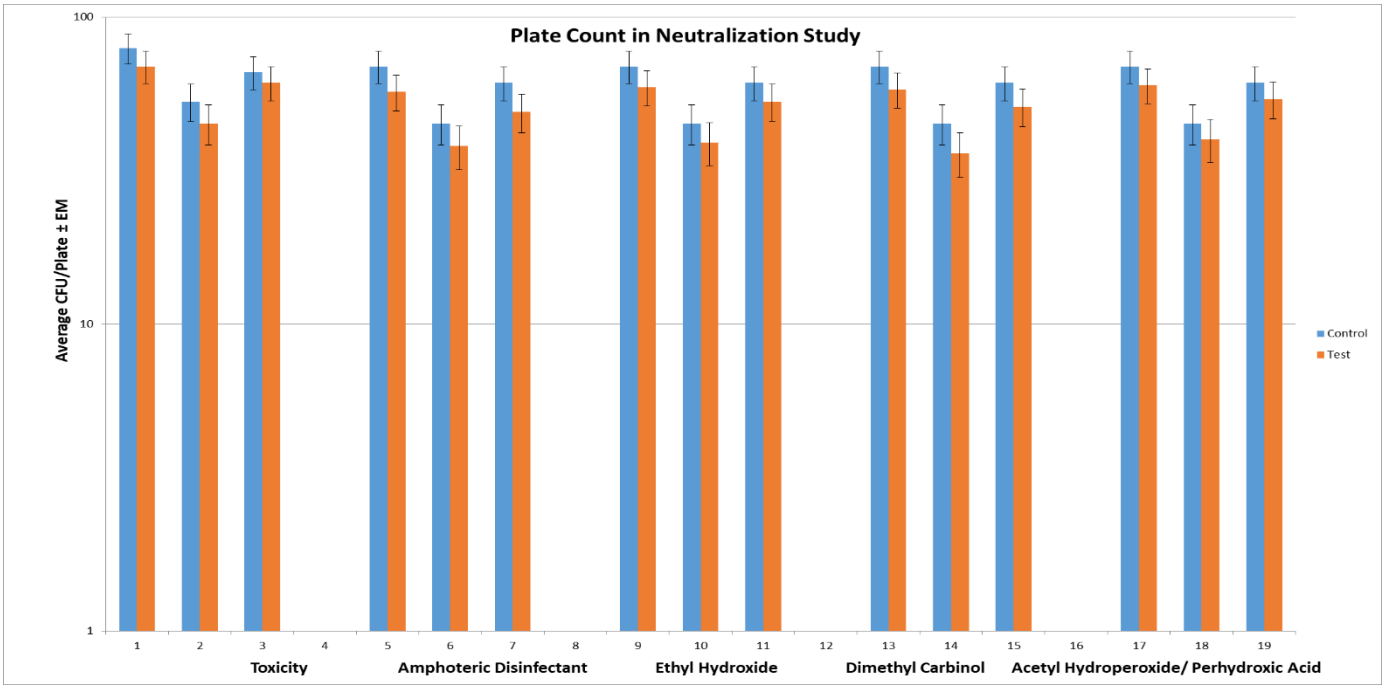

Fig. 3. Neutralization validation study showing the recovery of the test groups against controls. (EM: Error percent of the mean plate count expressed as CFU)

3.2. Analysis of the relative recovery between neutralization treatments

One-Way ANOVA test ($P < 0.05$) conducted on the relative bacterial recovery for three subjects over five treatments, showed significant difference between groups with exact P value of 0.0151 and the statistic value of 9.867. Table 1 demonstrated the recovery of microbial plate counts as transformed data of CFU with Standard Deviation (SD) and acceptable Relative Standard Deviation (RSD < 2%) were shown. The relative microbial recovery ± SD of all test groups was demonstrated in Fig. 4.

Table 1. Preliminary neutralization study design evaluation for selected biocidal agents to be used in a healthcare facility

Experiment Ω	Viability Control	Toxicity Group	Recovery ¥	Mean Recovery
I	1.90	1.84	0.969	0.970
II	1.72	1.65	0.959	SD = 0.011
III	1.82	1.79	0.981	RSD =1.157 %
Experiment Ω	Efficacy Control	Amphoteric Disinfectant Group £	Recovery ¥	Mean Recovery
I	1.84	1.76	0.955	0.952
II	1.65	1.58	0.956	SD = 0.005
III	1.79	1.69	0.947	RSD = 0.518 %
Experiment Ω	Efficacy Control	Ethyl Hydroxide Group	Recovery ¥	Mean Recovery
I	1.84	1.77	0.963	0.964
II	1.65	1.59	0.962	SD = 0.002
III	1.79	1.72	0.966	RSD = 0.188 %
Experiment Ω	Efficacy Control	Dimethyl Carbinol Group	Recovery ¥	Mean Recovery
I	1.84	1.76	0.959	0.952
II	1.65	1.56	0.941	SD = 0.010
III	1.79	1.71	0.956	RSD = 0.999 %
Experiment Ω	Efficacy Control	Acetyl Hydroperoxide/ Perhydroxic Acid Group €	Recovery ¥	Mean Recovery
I	1.84	1.78	0.967	0.969
II	1.65	1.60	0.969	SD = 0.002
III	1.79	1.73	0.970	RSD = 0.175 %

¥ An acceptance criterion was set to 0.938 which is equivalent to the recovery of three fourth of the control group count.SD: Standard Deviation.
Ω Individual experiments were performed in duplicates and the results were expressed as a logarithm of the average CFU/plate.RSD: Relative Standard Deviation.
£ The commercial product is basically composed of amines, n-C10-16-alkyltrimethylenedi-, as reaction products with chloroacetic acid.
€ The commercial product includes also acetic acid at 7.5 ± 2.5 % concentration.

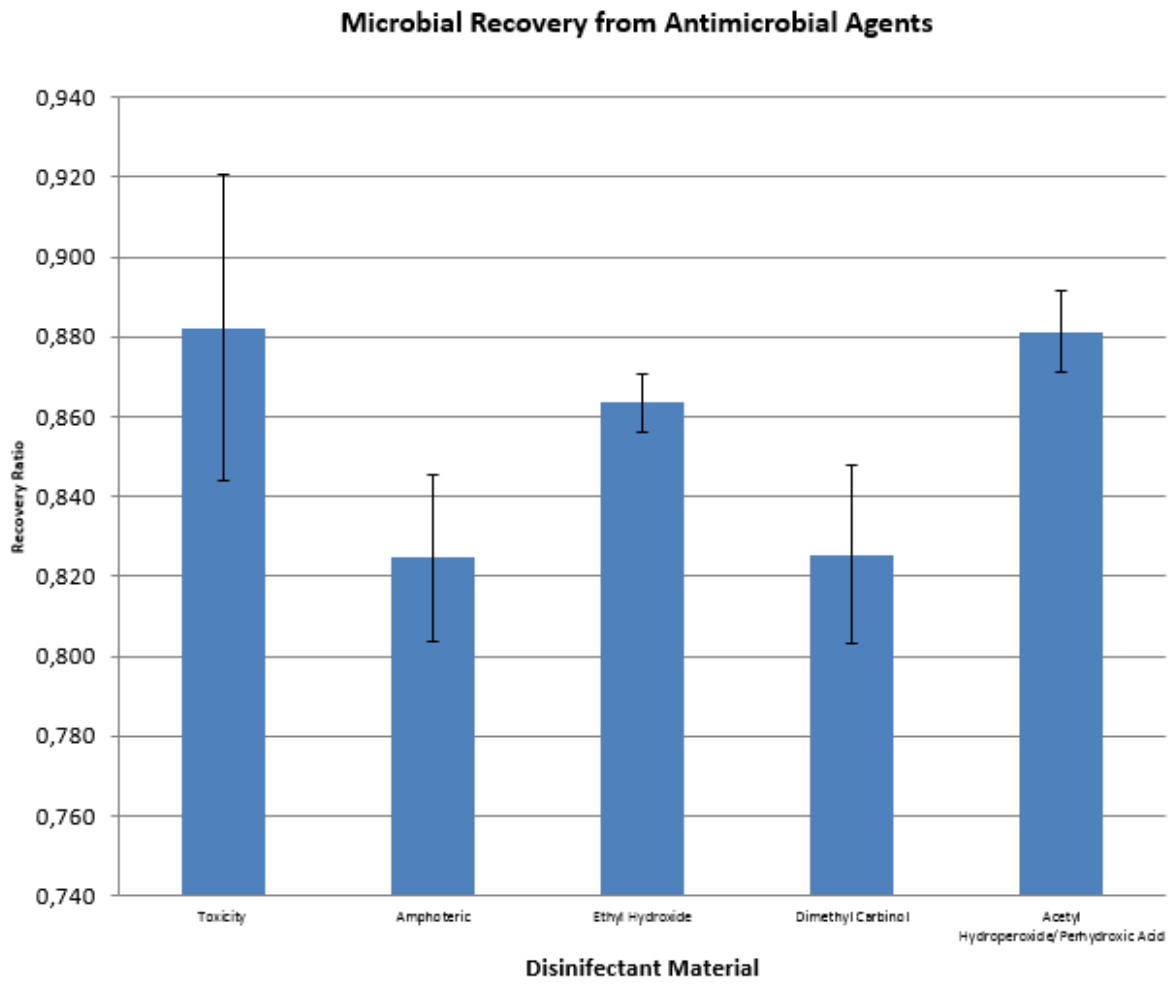

Fig. 4. *Burkholderia cepacia* complex (BCC) in the pooled result of the Neutralizer Toxicity (NT) and Neutralizer Efficacy (NE) tests along with standard deviation bars.

Multiple comparisons test between the values of the relative recovery of the original raw data did not reveal significant difference between groups at $\alpha = 0.05$ for five treatments (columns) and three subjects (rows). The Δ rank sum of rank sum (I – II) with adjusted P values for toxicity and efficacy groups of TX, AD, EH, DC and AP were AD vs. TX -9 (4 – 13) 0.2018, EH vs. TX -3 (10 -13) > 0.9999, DC vs. TX -8 (5 – 13) 0.3892, AP vs. TX 0 (13 – 13) > 0.9999, EH vs. AD 6 (10 – 4) > 0.9999, DC vs. AD 1 (5 – 4) > 0.9999, AP vs. AD 9 (13 – 4) 0.2018, DC vs. EH -5 (5 – 10) > 0.9999, AP vs. EH 3 (13 – 10) > 0.9999 and AP vs. DC 8 (13 – 5) 0.3892.

3.3. Pairwise analysis for the source of significance in microbial recovery between groups

Variance analysis ($P < 0.05$) of the plate count data showed exact P value < 0.0001 with the statistical value of 14.62. Multiple comparison was conducted for pairwise investigation of the treatments for the source of this variation at $\alpha = 0.05$ and the comparison included Test (T) against Control (C). The examination was conducted by calculating rank sum I from the first group minus Rank sum II from the second comparison to yield rank sum difference with P value was computed. The only significant difference was found between the amphoteric detergent test and the toxicity control group with adjected P value 0.0339 and the mean rank difference of (4 - 18) = -14.

3.4. Antibacterial activity of disinfectants over the study period

Biocidal activity examination over five minutes period showed more than 10,000 times reduction of the microbial population using surface contact test (Table 2). The average reduction factor for AD, EH and AP for wall, floor and metallic surfaces – pooled for the two test time periods of fresh and stored biocidal agents - exceeded 1.42×10^5, 1.50×10^5 and 1.68×10^5, respectively. Thus, the mean logarithmic reductions were > 5.14, > 5.17 and > 5.21 with (maximum – minimum) range of (5.36 – 5.00) and estimated approximate standard errors of 0.048, 0.045 and 0.055, respectively. The average reduction factor for DC for wall, floor and metallic surfaces – pooled for the two test time periods of fresh and stored biocidal agents - was 2.14×10^4, 2.03×10^4 and 6.32×10^4, respectively. Thus, the logarithmic reductions were 4.32, 4.30 and 4.79 with estimated standard errors of 0.043, 0.035 and 0.034, respectively. The mean ± standard deviation of the difference in the logarithmic microbial count reduction of the three experiments for wall, floor and metallic surfaces for DC was 0.029 ± 0.016, 0.045 ± 0.037 and 0.106 ± 0.22, respectively. The variation in the log reduction of bioburden

between wall and floor materials was insignificant being in the range of 0.007 to 0.032. While the microbial reduction from metallic (Stainless Steel) surfaces was considered significant compared to wall and floor structures. The reduction factor ± SD of the metallic surface versus both wall and floor materials in the triplicate was (0.447 ± 0.053, 0.474 ± 0.10, 0.486 ± 0.026) and (0.479 ± 0.048, 0.492 ± 0.009, 0.512 ± 0.028), respectively.

Table 2. Disinfectant validation using surface contact test on representative materials from the common surfaces found in the healthcare facility

Biocidal Agent£ / ¥CFU Recovery	Control Count			Freshly Prepared Disinfectant			Prepared Stored Disinfectant for 313 to 359 hours€		
	Coupon Material Mean Recovery (RSD%)			Coupon Material Mean Recovery (SE)			Coupon Material Mean Recovery (SE)		
I	PC	FL	MT	PC	FL	MT	PC	FL	MT
AD	6.18 (1.62)	6.20 (0.62)	6.27 (0.26)	<1	<1	<1	<1	<1	<1
EH				<1	<1	<1	<1	<1	<1
DC				1.81 (0.90)	1.85 (0.92)	1.36 (0.68)	1.83 (0.91)	1.88 (0.94)	1.46 (0.73)
AP				<1	<1	<1	<1	<1	<1
II	PC	FL	MT	PC	FL	MT	PC	FL	MT
AD	6.04 (0.93)	6.07 (1.70)	6.09 (2.05)	<1	<1	<1	<1	<1	<1
EH				<1	<1	<1	<1	<1	<1
DC				1.78 (0.89)	1.80 (0.90)	1.30 (0.65)	1.76 (0.88)	1.85 (0.92)	1.34 (0.67)
AP				<1	<1	<1	<1	<1	<1
III	PC	FL	MT	PC	FL	MT	PC	FL	MT
AD	6.20 (1.88)	6.22 (1.76)	6.28 (1.77)	<1	<1	<1	<1	<1	<1
EH				<1	<1	<1	<1	<1	<1
DC				1.83 (0.91)	1.89 (0.94)	1.43 (0.71)	1.85 (0.92)	1.90 (0.95)	1.52 (0.76)
AP				<1	<1	<1	<1	<1	<1

PC: Partition Construct, FL: Floor Lining, MT: Metal Tool, SE: Standard Error, RSD: Relative Standard Deviation
¥ Results of the plate count were expressed as logarithmically transformed results to the base ten
£Amphoteric Detergent, Ethyl Hydroxide, Dimethyl Carbinol and Acetyl hydroperoxide/Perhydroxic acid (abbreviated as AD, EH, DC and AP, respectively)
€ Storage was done at room temperature under ordinary working conditions

4. Discussion

The newly emerging Gram-negative bacterium is of significant health concern in healthcare facilities, notably hospitals (24). While BCC imposes little concern on healthy individuals, it demonstrated infection risk in hospitalized patients with defective health issues such as weak immunity and lung diseases (24, 25). Hence, the biochemically identified microorganism was included in the dynamic library database of the critically important environmental isolates. As a part of GxP in healthcare plants, regular examination of the efficacy of the sanitizers against the microbial isolates in the organization would be a critical task as an integral part of controlling nosocomial infections and minimization of cross-contamination risk, in addition to the Surgical Site Infections (SSI) issues (26). The disinfection protocol involved a spectrum of the biocidal agents in an application rotation program that minimizes the problem with prolonged use of each one (27). Materials, tools and equipment's corrosion and exposure toxicity are among the main mitigated challenges, in addition to the possible minimization of the antimicrobial resistance risk using the biocidal rotation concept.

To assess the antimicrobial activity of the disinfectants correctly, a complete stoppage of the biocidal action after a predefined contact period must be ensured (28). This was ensured using a combination of both dilution 1:10 (v/v) and chemical neutralization (28). To validate the neutralization technique, two comparisons analyses must be made (29). The first requirement is the proof of non-toxicity of the neutralizer on the test microbe. The second criterion is the evidence of the effectiveness of the neutralization process through the absence of evidence of suppression of microbial growth after mixing the neutralizer with the disinfectant agents at the working concentration (29). Each study involved its own control group as viability (microorganism) treatment for toxicity study and the toxicity (microorganism plus neutralizer) group for neutralizer efficacy. It should be noted that the microbial toxicity might originate from the neutralizer ingredients, disinfectant components (from its trace amounts, if complete neutralization was not ensured) and/or the byproduct of the chemical neutralization process due to neutralizer-biocide interaction (28,30-32). The selected microbial count range for the serial dilution of > 40 to 100 CFU/plate was selected as an optimal bioburden range to minimize count error.

Statistical inference was performed at different stages. The first one was broadly based on the initial examination of the recovery ratio that should meet the acceptance criterion (22). The second one gets it deeper into investigating the significance of the difference between the found results from the reference lower acceptance value and the upper microbial count result of the control (33). By virtue of the non-Gaussian nature of the microbiological distribution, the Wilcoxon signed-ranks test was used as a non-parametric that is equivalent to the paired t-test (34). It is most widely used to investigate for a difference in the median or mean of recorded

data - whether measurements were based on pairing examination or before and after readings on the same test subject (35). Also, it could be applied as a one-sample test to determine whether a particular sample was raised from a population with a predefined median value such as 0.75 recovery ratios of the raw data for CFU count/plate or logarithmically transformed figure for this microbial count of 0.938 as an acceptance criterion. In the present study, the microbial recovery ratios had met the acceptance criterion without significant difference from the control values.

The variations of the bacterial recoveries between different groups were examined using multiple comparison analyses. Interestingly, the detailed one-by-one comparison did not mark any significant difference at $\alpha = 0.05$. Nevertheless, the ANOVA test pinpointed a significant difference ($P = 0.05$). A situation that has been observed statistically in other previous works (36). Treatments were segregated as columns of CFU/plate recovery for individual group examination. A remarkable difference was detected to be possibly stemmed from the variation between the control plate count and the AD biocidal group. This is even though fact that this disinfectant did not fail apparently in the other preliminary statistical examination. Accordingly, a decision has been made to proceed in the testing procedure with disinfectant.

A disinfectant evaluation was executed by mimicking the application in the actual use – including storage time – on the most common surface materials that could be found in the building (37). These surfaces were made in the form of coupon shapes from the lining of the floor, wall material and metallic objects (mostly steel) (38). The surface contact was used instead of the suspension test because it is more realistic and challenging. This is because the microbial particles might be protected from the microscopic irregularities of the hard objects that hinder the full exposure of the cells to the biocidal environment in contrast to the suspended microbial cells where the surface area of exposure could be maximized accelerating the effect of the antimicrobial compounds (30). All investigated disinfectant formulae were effective directly after the preparation and after two weeks of storage at the ordinary facility-controlled environment in a well closed container. Interestingly, freshly prepared DC showed very low microbial recovery which was not significantly different from that after about 336 hours. The more significant microbial reduction observed with the metallic surfaces compared with wall and floor materials, notably with DC disinfectant, could suggest the unfavorable survivability conditions for the microorganism on the metal with possible antimicrobial effect on the viable cells.

Finally, it could be concluded that the commercially used disinfectants in the healthcare facility were effective in killing a large population ($\geq 1 \times 10^6$ CFU/coupon) of the microorganism under study (BCC) using a challenging over-kill strategy to account for a high load of bioburden that could be inoculated on the surfaces during the traffic of a heavy workload (39 - 41). This could be ensured only after an effective neutralization plan to avoid any overestimation of the biocidal agent activity due to the crippling residual amount of the disinfectant after the proposed contact period (42). This trace amount of the antimicrobial components might halt the microbial growth in the agar media leading to an exaggerated impression of the true potency of the biocidal agents. After successful implementation of this study, other microorganisms could be included in the validation program after identification to build a growing database for better control of microbial dissemination and contamination. The result of the ampholyte antimicrobial product might require further extended investigation in another study in the future including a greater number of replicates to confirm or exclude the observed significant difference. The presence of a toxic trace level of antimicrobial constituents – that is not enough to block the full growth of the microorganism totally - cannot be ruled out. However, this would be needed to be addressed in a new study that would require deeper analysis. It is also advisable to select the materials of construction for both equipment, tools, furniture, wall and floor surfaces carefully. Implementation of smooth surface materials that possess antimicrobial properties in healthcare buildings will be an advantage in hygiene control policy in the organization.

Conflict of interest

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None to declare

Authors' contributions

Concept: D.E.E., E.R.R., Design: E.R.R., D.E.E., Data Collection or Processing: D.E.E., M.E.E., Analysis or Interpretation: M.E.E., Literature Search: E.R.R., Writing: M.E.E.

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