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Bacteriological and Fungal Evaluation of Three Brands of Disinfectants Sold in Calabar Municipality, Cross River State

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Authors' contributions

This work was carried out in collaboration between all authors. All the authors designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. All authors managed the literature searches and read and approved the final manuscript.

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ABSTRACT

The antimicrobial activity of three commonly used disinfectant brands D, R and P against *Escherichia coli, Staphylococcus aureus, Candida albicans* and *Pseudomonas aeruginosa* were investigated. Their efficacies were determined using the In-use test and capacity test (Kelsey-Sykes) at different dilutions of the test disinfectants while their potency were tested under clean and dirty conditions using the Kelsey-Sykes standard test. The results of the study show that as the concentration reduced the susceptibility rates of the test organisms were also reduced. The potency of the disinfectants showed that they were all effective, with D being the most potent

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disinfectant as compared to other brands (R and P) under study. Furthermore, *Pseudomonas aeruginosa* was more resistant than other test organisms. To ensure efficacy and maintain standard, regular tests should be carried out on old and new disinfectant products.

Keywords: Disinfectants; efficacy; microbial contamination; susceptibility.

1. INTRODUCTION

Millions of homes around the world, clinics, hospitals, offices, laboratories and schools have and use atleast one type of disinfectants that is used for disinfecting inanimate objects and surfaces. Disinfection is the reduction of the level microorganisms to a point that does not cause harm to humans or goods [1]. Disinfectants have long been identified as an effective means of controlling microbial populations and still remain an important control measure in the spread of infectious diseases [2]. Despite their continuous usage, infectious disease and their impact remains a significant problem in today's society [2]. Disinfectants range from pine oils, bromine, iodine, hypochloride, phenolics, alcohols to quaternary ammonium compounds. The type of the organisms controlled and the mechanism of action varies widely between these compounds. Most of them perforate cell walls of the microorganisms, allowing the contents to leak out, while a few of them penetrate the cell destroying the microorganism from within [3-6].

The widespread use of disinfectant products has brought about some speculations on the development of microbial resistance, most importantly cross-resistance between antibiotics and biocides [4,5]. According to Moorer et al. [6] disinfection does not always kill all microorganisms' especially resistant bacteria and have been observed to be less effective than sterilization. However, antimicrobial resistance is frequently conferred by plasmid or transposons, which have allowed rapid and extensive spread through the globe [4,5]. But the selection and controlled use of effective disinfectants have been advised since environmental surfaces as well as medical equipment can serve as vectors of infectious agents most especially for susceptible hosts in the hospital settings. Even with the enormous use of disinfectants, information on the efficacy of some these disinfectants are scarce.

This study is therefore aimed at evaluating the efficacy of three different brands of disinfectants sold in Calabar against a gram positive (*Staphylococcus aureus*), two gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria and a fungus (*Candida albicans*).

2. MATERIALS AND METHODS

2.1 Study Area

Calabar municipality lies on latitude $50^{o}32^{1}$ and $40^{o}22^{1}$ and longitude $70^{o}50^{1}$ and $90^{o}28^{1}$, respectively. Has a land of about 481sqkm^{2} and had a population of 371,022 as at the 2006 census [7].

2.2 Source of Microbial Isolates

The isolates used in this study were obtained from clinical samples such as wound swabs and vaginal swabs) from the University of Calabar Medical Center and taken the laboratory for microbiological analysis. This was done following standard microbiological procedures [8]. The isolates included *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*.

2.3 Disinfectants

The disinfectants used in this study were selected based on wide their acceptability and frequency of use in the hospitals, offices and homes. They were purchased in duplicates from dealers in Calabar, Cross River State of Nigeria. The disinfectants were coded as brands D, R and P.

2.4 In-use Test

This was carried out following procedures described by Akubueze et al. [9]. Briefly, into six tubes containing 9ml of nutrient broth each, 1ml each of the sampled disinfectants were introduced into them. Then, ten drops each of each of the diluted sample (disinfectant and nutrient broth) respectively were introduced into two plates each containing freshly prepared nutrient agar. Both plates were incubated differently, one at 37℃ for 72hours and the other at room temperature (25℃) for 7 days. After incubation, the plates were observed for growth.

2.5 Capacity Test of Kelsey-Sykes

This was carried out following procedures described previously [9] Briefly about 3-4 colonies of the test organism was inoculated into 10 ml nutrient broth for bacteria and sabouraud dextrose broth for fungi, and incubated at 37℃ for 24 hours. In the same vein, 3 different handkerchiefs used to mop laboratory benches were cut into 3 pieces each and soaked in 1 litre of tap water containing one cap full of the disinfectants, respectively and incubated for 24 hours. Subsequently, 3 mls each of the pure (clean) disinfectants as well as the dirty (suspensions containing water, disinfectant and dirty handkerchief) disinfectants were pipetted into different test tubes. At time intervals of 0, 10 and 20 minutes, 1ml of the standardized broth culture of the test organism was introduced into each of the test tubes containing the disinfectants respectively. After a contact period of 8, 18 and 28 mins, respectively, 0.02 ml of the disinfectant culture mixture was introduced into peptone broth in replicates. All the inoculated broth tubes were then incubated at 32℃ for 48 hours, after which they were examined for growth (turbidity). The tubes showing growth were recorded as positive while those without growth were assigned negative. Any disinfectant that scored 2 or more negatives out of a set of five replicates recovery tubes, after the first, second and 3rd challenges were assumed to have passed the test. The procedure was repeated on all the test isolates employed in this study.

2.6 Determination of Minimum Inhibitory and Bactericidal Concentrations (MIC, MBC) of Disinfectants against Test Isolates

The minimum inhibitory concentrations of disinfectants were determined using the arithmetic dilution method [10]. A series of increasing concentrations of the disinfectants were obtained using serial dilution method in which 5ml of distilled water was first introduced into each test tube and 5ml of the concentrated and then used to prepare dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, and 1:1024. Tubes containing distilled water were kept as the controls [2]. Exactly, 0.5 ml of the test organisms which had been inoculated in buffered peptone water for 24 hours were introduced into each test tube containing the disinfectants respectively and incubated at 37℃ for 24 hours and observed for growth. The dilutions that showed no visible growth or turbidity after incubation were considered the MICs for each disinfectant against each test isolate. Test tubes that showed no visible growth from the MIC were inoculated into freshly prepared nutrient agar plates and incubated at 37℃ for 24 hours. The concentration at which no growth was observed visibly from the plates was considered the MBCs.

3. RESULTS

The result of the In-use test is presented in Table 1. From the In-use test, less than five colonies were found on all plates held at room temperature. D showed 1 colony and no colony, R showed 3 and 2 colonies, respectively while P showed 4 and 2 colonies, respectively at the temperature of 37℃ after 3 days of incubation. However, the plates held at room temperature for 7 days showed no visible growth.

The results obtained for Capacity test of D, R and P against *Pseudomonas aeruginosa* is presented in Tables 2. Under clean conditions for D at concentrations of 1% and 0.81 passed but failed at 0.73 and at dirty conditions. R and P passed at 1.0% under clean conditions but failed at other concentrations and also under dirty conditions.

Table 1. In-use coefficient for different brands of disinfectant

Disinfectants brands	Batch no	Colonies at 3 days (37℃)	Colonies at for 7 days (25℃)	Interpretation
D1	DL376N	1	NG	No contamination
D2	DL609M	NG	NG	No contamination
R2	018	3	NG	No contamination
R2	069	2	NG	No contamination
P3	857	4	NG	No contamination
P3	838	2	NG	No contamination

Keys: NG = no growth

Table 2. Kelsey – Sykes test for disinfectant under clean and dirty conditions using *Pseudomonas aeruginosa*

Condition	Concentration	Challenge number			Result
D	**(%)**	**1**	**2**	**3**	
Clean	1.0	- - - - +	- - - - +	- - - - +	PASS
	0.81	- - - + +	- - + + +	- + + + +	PASS
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL
Dirty	1.0	+ + + + +	+ + + + +	+ + + + +	FAIL
	0.81	+ + + + +	+ + + + +	+ + + + +	FAIL
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL
R	1.0	- - - - +	- - + + +	- + + + +	PASS
Clean	0.81	+ + + + +	+ + + + +	+ + + + +	FAIL
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL
Dirty	1.0	+ + + + +	+ + + + +	+ + + + +	FAIL
	0.81	+ + + + +	+ + + + +	+ + + + +	FAIL
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL
P	1.0	- - + + +	- - + + +	- + + + +	PASS
Clean	0.81	+ + + + +	+ + + + +	+ + + + +	FAIL
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL
Dirty	1.0	+ + + + +	+ + + + +	+ + + + +	FAIL
	0.81	+ + + + +	+ + + + +	+ + + + +	FAIL
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL

Table 3. Kelsey – Sykes test for disinfectants under clean and dirty conditions using *Staphylococcus aureus*

Condition	Concentration	Challenge number			Result
D	**(%)**	**1**	**2**	**3**	
Clean	1.0	- - - + +	- - - + +	- - - + +	PASS
	0.81	- - - - +	- + + + +	- - + + +	PASS
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL
Dirty	1.0	- - + + +	- - - + +	- + + + +	PASS
	0.81	- + + + +	- + + + +	- - + + +	PASS
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL
R	1.0	- - - - -	- - - - -	- - - + +	PASS
Clean	0.81	- - + + +	- + + + +	- - + + +	PASS
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL
Dirty	1.0	- - - - +	- - + + +	- + + + +	PASS
	0.81	- - - - +	- - - + +	+ + + + +	PASS
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL
P	1.0	- - - + +	- - + + +	- + + + +	PASS
Clean	0.81	- - - - -	- - - + +	- - + + +	PASS
	0.73	- - + + +	- - + + +	- - - + +	PASS
Dirty	1.0	- - - + +	- - - + +	- - + + +	PASS
	0.81	- + + + +	- - + + +	+ + + + +	PASS
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL

Table 3 shows the capacity test for the disinfectant against *S. aureus.* For brand D and R, consistently only concentrated of 0.73 failed for both clean and dirty conditions. However, for brand P, all concentration passed at all concentrations.

Table 4 shows the capacity test for the E. coli under clean and dirty conditions. Only concentration of 1.0 passed the challenge and 0.81 and 0.73 failed for both D, R, and P under clean condition. However, P failed at all three concentrations under both challenge conditions.

Table 5 shows the Capacity test for all three disinfectants. Consistently, all three brands failed at concentration 0.73 but passed at other concentrations. However, brand R was the most effective at the highest concentration.

Table 4. Kelsey – Sykes test for D under clean and dirty conditions using *Escherichia coli*

Condition	Concentration	Challenge number			Result
D	**(%)**	**1**	**2**	**3**	
Clean	1.0	- - + + +	- - + + +	- + + + +	PASS
	0.81	+ + + + +	+ + + + +	+ + + + +	FAIL
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL
Dirty	1.0	- - - + +	- + + + +	+ + + + +	PASS
	0.81	+ + + + +	+ + + + +	+ + + + +	FAIL
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL
R	1.0	- - - - -	- + + + +	- - + + +	PASS
Clean	0.81	+ + + + +	+ + + + +	+ + + + +	FAIL
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL
Dirty	1.0	- - - - -	- - + + +	+ + + + +	PASS
	0.81	+ + + + +	+ + + + +	+ + + + +	FAIL
P	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL
Clean	1.0	- - - - +	- - + + +	- + + + +	PASS
	0.81	+ + + + +	+ + + + +	+ + + + +	FAIL
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL
Dirty	1.0	+ + + + +	+ + + + +	+ + + + +	FAIL
	0.81	+ + + + +	+ + + + +	+ + + + +	FAIL
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL

Table 5. Kelsey – Sykes test for D under clean and dirty conditions using *Candida albican*

Condition	Concentration	Challenge number			Result
D	**(%)**	**1**	**2**	**3**	
Clean	1.0	- + + + +	- - + + +	+ + + + +	PASS
	0.81	- - + + +	- + + + +	+ + + + +	PASS
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL
Dirty	1.0	- - - - +	- - - + +	- - + + +	PASS
	0.81	- - + + +	- + + + +	+ + + + +	PASS
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL
R	1.0	- - - - -	- - - - -	- - - - +	PASS
Clean	0.81	- - - - +	- - - - +	+ + + + +	PASS
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL
Dirty	1.0	- - - - -	- - - - -	- - - - +	PASS
	0.81	- - - + +	- - + + +	+ + + + +	PASS
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL
P	1.0	- - - - -	- - - - +	- - + + +	PASS
Clean	0.81	- - + + +	- - - + +	+ + + + +	PASS
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL
Dirty	1.0	- - - + +	- - - + +	- - - - +	PASS
	0.81	- - + + +	- + + + +	+ + + + +	PASS
	0.73	+ + + + +	+ + + + +	+ + + + +	FAIL

A wide divergence was observed among isolates in their response against the disinfectants at different concentrations as shown in Table 6 below. The MIC/MBC of D against *Pseudomonas aeruginosa* and *Escherichia coli* was 1:64 and 1:32 respectively while that of *Staphylococcus aureus* was 1:32 and 1:16 respectively. However, MIC/MBC of *Candida albicans* was observed to be 1:256 and 1:128 respectively. Also, the MIC/MBC of Rt against *Pseudomonas aeruginosa* and *Candida albicans* was observed to be 1:32 and 1:16 while that of *Staphylococcus aureus* and *Escherichia coli* was 1:128 and 1:64 respectively. However, the MIC/MBC of P against *Pseudomonas aeruginosa* and *Escherichia coli* was observed to be 1:64 and 1:32 respectively while that of *Staphylococcus aureus* was 1:256 and 1:128. Meanwhile the MIC/MBC of *Candida albicans* was observed to be 1:52 and 1:1024 respectively as shown in the Table 6.

Table 6. The MIC and MBC of the disinfectants against test isolates

Disinfectant	Organism	MIC	Control	MBC	Control
D	*Pseudomonas aeruginosa*	1.64	0.00	1.32	0.00
	Staphylococcus aureus	1:32	0.00	1:16	0.00
	Escherichia coli	1:64	0.00	1:32	0.00
	Candida albicans	1:256	0.00	1:128	0.00
R	*Pseudomonas aeruginosa*	1:32	0.00	1:16	0.00
	Staphylococcus aureus	1:128	0.00	1:64	0.00
	Escherichia coli	1:128	0.00	1:64	0.00
	Candida albicans	1:32	0.00	1:16	0.00
P	*Pseudomonas aeruginosa*	1:64	0.00	1:32	0.00
	Staphylococcus aureus	1:256	0.00	1:128	0.00
	Escherichia coli	1:64	0.00	1:32	0.00
	Candida albicans	1:512	0.00	1:256	0.00

4. DISCUSSION

The potency characteristics of the test disinfectant against test organisms (*Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Candida albicans*) were investigated in this study. All the disinfectants as observed using the In-use test were pure (clean) prior to use and this is contrary to earlier studies [10,11], where it was observed that all disinfectants were contaminated prior to use. However, to ensure effectiveness of disinfectants, it is necessary to evaluate new disinfectants before use in homes and hospitals.

The microbial inhibition and killing effect of these disinfectants were observed to be increasing as the concentration of the disinfectant were increased. This conforms to the study by [12] and [13] where they observed that the higher the concentration of the solution, the more potent and effective the solution will be. The result of the capacity test showed that non-sporulating, non-mycobacterial and gram positive organisms were more susceptible to disinfectants than Gram negative organisms. This observation is in line with the research done by [14] which showed that Gram negative organisms were more resistant to disinfectants than Gram positive bacteria generally because the outer membrane of gram negative bacteria acts as a barrier that limits the entry of many chemically unrelated types of antimicrobial agents [4]. The gram positive organisms like *Staphylococcus aureus* on the other hand are usually seen as the most susceptible even when some strains possess capsule. This may be due to the peptidoglycan and teichoic acids components of their cell walls. Though, the capsule exhibit some level of resistance to antimicrobial agents but where considerable resistance is encountered in this group of organisms, it is often assumed to have been acquired most especially through mobile genetic elements such as plasmids and transposons.

Despite the inhibitory concentration of D on *Pseudomonas aeruginosa* and *Escherichia coli*, these organisms still showed susceptibility at the concentration of 1:32 compared to *Staphylococcus aureus* and *Candida albicans* which shows susceptibility at 1:16 and 1:128, respectively. However, *Pseudomonas aeruginosa* and *Candida albicans* showed a more reduced susceptibility (1:16) to R disinfectant. This clearly showed that R has a less bactericidal effect on *Pseudomonas aeruginosa* and *Candida albicans* compared to D. Also, *Escherichia coli* and *Staphylococcus aureus* were more susceptible to R at different concentrations, even to the dilution of 1:128 as compared to other microbes. However, Gram positive organisms (*Staphylococcus aureus* and *Candida albicans*) were found to be more susceptible to P at different concentrations even at the dilution of 1:256 and 1:512 respectively.

Among the various disinfectants employed in this study, D was observed to be more effective in killing the test organisms followed by R and lastly P, this conform with the work done by [15] that D was effective in killing even Gram negative organisms. this may be due to differences in the activity of the disinfectants, mode of actions, the media components as well as active components, which may have affected the activity outcome of activity testing since the presence of organic matter have been observed as a major factor that affects the action of disinfectants [16].

The disinfectant coefficient observed using capacity test method compared favourably with standard phenol and seem to be more appreciated because this test simulate natural conditions due to the addition of soiled handkerchief in water/disinfectant/organic matter solution. However, since research have linked the contamination of disinfectants in homes and hospital environments to sub-optimal sanitary practices during preparation and distribution, the addition of residual amount of disinfectants during use in homes and hospital environments could contribute to selection and maintenance of multi strains of organisms [10].

5. CONCLUSION

This study emphasizes the need for hospitals as well as homes to imbibe standard disinfection policy and procedures that ensure proper use of disinfectants and antiseptics since use of inadequate (sub-optimal) concentration of disinfectants have been implicated in the development of resistant and virulent strains of organisms. This study has confirmed that D was more effective against organisms followed by R and lastly P.

6. RECOMMENDATIONS

In view of the fear of the development of resistance by microorganisms exposed to disinfectants. It is necessary that clean (pure) preparations of disinfectants be used routinely. In addition, the dilution of the disinfectant should be based on concentration ranges that have been confirmed to be effective against organisms. Also, a toxicity study of active ingredients in disinfectants should be ascertained to protect users. Finally, newly manufactured disinfectants should be routinely tested to ensure they are in good condition.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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