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Antibacterial Efficacy of the In-Use Dilutions of Common Disinfectants against *Pseudomonas aeruginosa* Isolates in a Tertiary Care Hospital in Calabar, Nigeria

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Abstract:
Background: *Microorganisms are readily transmitted through various means including contaminated materials or instruments used in patient care. Disinfection is an important component of infection control. Use of chemical disinfectant has led to drastic reduction in transmission and spread of hospital pathogens. Concentration has a great influence on the potency of disinfectants. An evaluation study to determine the effectiveness of in-use dilutions of common disinfectants used in the University of Calabar Teaching Hospital on Pseudomonas aeruginosa strains was carried out.*
Method: *P.aeruginosa isolates were obtained from inanimate sources in the hospital and characterized with a Gram Negative-ID system using P.aeruginosa ATCC 27853 as control. In-use dilutions of four disinfectants, Dettol, izal, savlon and bleach, were obtained from house-keeping staff in the hospital wards. The Rideal-walker phenol coefficient test was used to determine disinfectant efficacy while thequantitative suspension test was used for their antimicrobial evaluation.*
Results: *Izal had the highest phenol coefficient of 3.25 savlon, bleach and Dettol had phenol coefficient of 2.75, 0.75 and 0.50 respectively. Savlon eliminated all viable cells of P.aeruginosa after five minutes exposure.Izal eliminated all cells after 20 minutes while Dettol and bleach had similar activities and eliminated cells after 15 minutes exposure.*
Conclusion: *The in-use dilution of izal was ineffective although it had the highest phenol coefficient of the four disinfectants tested. The concentrations of in-use solutions of the three disinfectants should be reviewed to enhance their usefulness in infection prevention and control in the hospital.*
Key words: *Disinfectants, antibacterial activity, infection control*

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I. Introduction

Healthcare associated infections (HAIs) are those infections that are acquired as a result of giving or receiving care in health facilities. It is a leading cause of morbidity and mortality worldwide. The hospital environment is conducive for the proliferation and spread of infectious agents because it houses sick people who habour disease agents, some of them very sick that they become immunocompromised and can readily acquire these agents.(1) Patient care involves several activities, some invasive, and may encourage the transfer of pathogens from one person to another, especially with contaminated instruments.(2) The situation is worse where infection control is not implemented with the commitment it deserves.*Pseudomonas aeruginosa*is frequently found in hospital environments, particularly the ICUs.(3)It is an important agent causing a variety of infections which account for about 10% of HAIs in the United States.(1,4,5)

This organism is the epitome of an opportunistic pathogen of humans, exploiting breaks in the host defences to initiate infection.(4) It is known to be innately resistant to many antimicrobial agents. The success of this organism as a pathogen is largely due to the production of a myriad of virulence factors and its tendency to colonize surfaces in an intractable biofilm form which makes the cells impervious to therapeutic concentrations of antibiotics.(5) It also possesses a remarkable array of physiological traits that may contribute to its pathogenicity.(6) Moreover, it maintains antibiotic resistance plasmids and is able to receive and transfer same from and to other bacteria with which it lives as normal flora, by means of the bacteria mechanism of horizontal gene transfer (HGT), mainly transduction and conjugation.(6)*P. aeruginosa*has been reported to survive even in disinfectant solutions.(7)

Since microorganisms were recognized as agents of infection, various methods have been used either to destroy them completely or reduce their population.[8] The concept of decontamination or disinfection forms a basic component of any infection control strategy. Disinfection is defined as the selective elimination of certain undesirable organisms in order to prevent their transmission.[9] One of the early methods involved was the use of chemicals disinfectants. Disinfectants are used in hospitals for the general disinfection of surfaces and cleaning of equipment and instruments.[10]Onaolapo[11] reported that concentration has a great influence on the potencyof disinfectants, that is, a bactericidal disinfectant may become bacteriostatic at lower concentration, although microorganisms differ in their sensitivity to chemical germicides and salts.[12] Some researchers[13] have reported the survival in and contamination of working dilutions of some disinfectants in hospitals by microorganisms. This study was designed therefore to evaluate the antibacterial activities of the most commonly used disinfectants in the University ofCalabar Teaching Hospital (UCTH), Calabar, Nigeria on *P. aeruginosa* isolates.

II. Materials And Methods

Specimen collection and processing

The specimens used in this study were obtained by swabbing the surfaces of inanimate objects in some wards of the University ofCalabar Teaching Hospital (UCTH). They included 10 floor swabs, 5 bedding swabs, 5 drug dispensing tray swabs, 5 sink/drain swabs and 5 water storage container swabs. These were inoculated on Pseudomonas isolation agar (PIA) and incubated overnight at 37^0C. The isolates were characterized using the Microgen GN A+B-ID system (MicrogenBioproduct, UK) which employs 24 standard biochemical substrates in microwells. All positive reactions were recorded with the aid ofcolour chart provided. Identification of organisms was done with *P. aeruginosa*ATCC 27853 as control. On theMicrogen GN A+B-ID report form, the substrates were organized into triplets (sets of 3 reactions) with each substrate assigned a numerical value (1, 2 or 4). The sum of the positive reactions for each triplet formed a single digit of the Octal code that was used to determine the identity of the isolate. The Octal code was entered into the Microgen identification system software (MID-60 Ver 1.2.5.26), which generated a report of the five most likely organisms in the selected database.

One hundred and ten health care workers (HCWs) were provided with questionnaires and their responses provided data on the most commonly used disinfectants in the hospital. The first four disinfectantswere selected for evaluation; their in-use dilutions were obtained from housekeeping staff in the wards.

Phenol coefficient test

The Rideal-Walker phenol coefficient test was used to determine the efficacy of the disinfectants.Approximately 5ml of sample containing five serial dilutions of phenol and test disinfectants (1:100, 1:150, 1:200, 1:250 and 1:300) were inoculated with *Staphylococcus aureus*. At 5, 10 and 15 minutes intervals, 0.1ml sample of the dilutions were withdrawn and transferred into fresh nutrient broth and incubated at 37^0C for 24-48 hours. The phenol coefficient was determined as the ratio of the reciprocal of the highest dilution of disinfectant that prevented growth, at 10 minutes and not 5 minutes, to that of phenol.

Quantitative suspension test of chemical disinfectants

The quantitative suspension test for antibacterial evaluation of various disinfectants in timed experiment was used,[13] 2.5ml of the overnight broth culture of *P.aeruginosa* isolate was mixed with 37.5ml of each disinfectant solution yielding 40ml of the in-use dilution containing viable cells. For control, 25ml of the cell suspension was added to 37.5ml of sterile water. The bacteria count in the solution at zero time was $1x10^6$CFU/ml. after exposure time of 5, 10, 15, 20 and 30 minutes, 4ml of suspension was withdrawn and added to 9ml of neutralizer (0.5% tween 80 in nutrient broth), mixed thoroughly and left to stand for one minute in order to inactivate any residual disinfectant. Then 0.1ml of the suspension was inoculated on nutrient agar plate in duplicates and incubated at 37^0C for 24 hours. The colonies on the plates were counted thereafter and calculated to give cfu/ml. The control did not contain the disinfectant, but only serially diluted cell suspension was plated and counted.

III. Results

Of the 30 swab specimens examined in this study, 12 yielded *P.aeruginosa*strains. Six (50.0%) were from floor swabs while sinks/drains, water storage containers and drug dispensing trays yielded 3(25.0%), 2(16.7%) and 1(8.3%) isolates respectively (Table 1). The most commonly used disinfectant, according to 110 HCWs' responses, were bleach 32(29.1%), izal 22(20.0%), savlon 18(16.4%), dettol 8(7.27%), methylated spirit 5(4.6%) and detergent 2(1.8%) while 23(20.9%) of respondents did not specify (Table 2). The phenol coefficients of the first four commonly used disinfectantsin the hospital were as follows;saponified cresol (Izal)

had the highest phenol coefficient of 3.25 followed by chlorhexidinegluconate (savlon), hypochlorite (bleach) and dichloroxylenol (dettol) with phenol coefficients of 2.75, 0.75 and 0.50 respectively (Table 3). The bactericidal activity of the in-use dilutions of each of these disinfectants on *P. aeruginosa*was also assessed. The result of the quantitative suspension test is presented in Table 4. *P. aeruginosa*was not susceptible to some of the disinfectants tested. Izal was ineffective against the cells at five minutes, 10 minutes and 15 minutes of exposure, however, after 20 minutes all the cells were eliminated. Bleach had similar activity like dettol as all cells were totally destroyed after 15 minutes exposure. Savlon was the most effective disinfectant on *P. aeruginosa*as all the cells were eliminated after 5 minutes interaction time.

IV. Discussion

The use of disinfectants and antiseptics constitutes an important component of infection control in hospitals.[14] This practice, initiated by Robert Koch, Louis Pasteur and Joseph Lister in the 19th century, has proved to be lifesaving and has led to the drastic reduction in the transmission and spread of HAIs. Analysis of the most commonly used disinfectants (dettol, izal, savlon and bleach) in the study hospital showed that savlon had the greatest antibacterial activity against *P. aeruginosa* strain tested. The disinfectant eliminated all viable cells after only 5 minutes of exposure. Both dettol and bleach had similar but lower activity than savlon and were only able to eliminate the organism after 15 minutes exposure. Izal was ineffective (Table 4). Ballows[15]opined that concentrations, active ingredients of antiseptics/disinfectants and condition of storage affect the potency and effectiveness of these chemicals. Chlorhexidine (savlon) has been used to successfully decrease the burden of MDR-GNB onpatients' skin and HCWs' hands and inanimate surfaces.[16] In separate studies in Abuja and Port Harcourt, Nigeria,savlon was found to be the most effective disinfectant against the different organisms tested.[15, 16]*P. aeruginosa*is said to be able to grow in almost any environment and has been shown to survive for more than a year under certain conditions.[17] It can survive on a dry floor for up to five weeks.[18] Other reservoirs include medical and respiratory equipment, sinks and mops and transmission may occur through direct patient contact with contaminated reservoirs. In this study, the highest number of isolates were from hospital floors, 6(50.0%) followed by sinks and drains 3(25.0%) and saponified cresol (izal) which was one of the most commonly used disinfectants for cleaning floors and surfaces (Tables 2 and 3) was found to be the least effective against *P. aeruginosa*isolates tested. These results provide information which should serve as a guide in the selection and appropriate application of these chemical agents for disinfection, an imperative in the prevention and control of HAIs. This is all the more urgent, even as we face the scourge of the deadly Lassa fever and Ebola virus disease (EVD) in the country and the continent. It is therefore necessary to review the concentrations of working solutions of disinfectants in our hospital in order to achieve the desired objectives of infection control.

V. Conclusion

The challenge of HAIs is a global phenomenon.*P. aeruginosa*is the most common gram-negative bacterium found in HAIs. The use of chemical disinfectants in hospitals plays a major role in reducing the spread of infections. This study has shown bleach, izal, savlon and dettol to be the most commonly used disinfectants in UCTH, of which the in-use dilution of savlon was found to have the highestantibacterial activity against*P. aeruginosa.* Izal was not effective although it had the highest phenol coefficient. The in-use concentration of working solutions of disinfectants is vital to achieving the purpose of disinfection, which is infection control.

Table 1. Distribution of *P. aeruginosa*isolates according to inanimate source of specimen

Source No. of specimen	**No. of isolates (%)**	
Floor	10	6(50.0)
Beddings	5	0(0.0)
Drug dispensing tray	5	1(8.3)
Sinks/drains	5	3(25.0)
Water storage	5	2(16.7)
Total	**30**	**12(100)**

Table 2. Most common disinfectants used by HCWs in the study hospital

Disinfectant	**No. of respondents (%)**
Izal	22(20.0)
Dettol	8(7.3)
Bleach	32(29.0)
Detergent	2(1.8)
Savlon	18(16.4)
Methylated spirit	5(4.6)
Not specified	23(20.9)
Total	**110 (100)**

Table 3. Phenol coefficient of test disinfectants

Disinfectant	**Phenol coefficient**
D1 (izal)	3.25
D2 (dettol)	0.50
D3 (bleach)	0.75
D4 (savlon)	2.75

Table 4. Antibacterial activity of test disinfectant (in-use dilution) against *P. aeruginosa*

Disinfectant	colony counts after minutes (viable cells/ml)				
	5	10	15	20	30
D1	$2.3x10^2$	$1.5 x 10^2$	$5.0 x 10^1$	0	0
D2	$2.0 x 10^1$	$1.0 x 10^1$	0	0	0
D3	$5.0 x 10^1$	$2.0 x 10^1$	0	0	0
D4	0	0	0	0	0
Control	$1.0 x 10^6$	$1.0 x 10^6$	$1.0 x 10^6$	$1.0 x 10^6$	$1.0 x 10^6$

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