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Evaluation of Effectiveness of Three Commonly Used Disinfectant on Clinical Isolate of *Pseudomonas Aeruginosa*

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ABSTRACT

This research was designed to evaluate the effectiveness of three commonly used disinfectants on clinical isolate of *Pseudomonas aeruginosa* using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Pseudomonas aeruginosa*. Isolates of *Pseudomonas aeruginosa* were collected from Ondo State Trauma and Surgical Center, Ondo. The identity of the isolate was confirmed using cultural, morphological and biochemical characteristics. Three major disinfectants namely Jik(Sodium Hypochlorite), Dettol(Chloroxylenol) and Izal were evaluated on their effect on *Pseudomonas aeruginosa*. The effect of percentage weight per volume (%w/v) of the chemical composition of each of the disinfectants were determined on the isolate of *Pseudomonas aeruginosa* using MIC and MBC. A serial concentration of 0.125%w/v to 35%w/v of the chemical composition of the disinfectant was made. The antibiotic susceptibility of the isolate was determined by discs diffusion method as well. The minimum inhibitory concentration MIC of Jik was found to be 0.5%w/v and MBC was 1.0%w/v while the MIC and MBC of Dettol was 1.5 and 2.0%w/v respectively. Izal had MIC of 5% w/v and the MBC of 10.0%w/v. Izal showed the lowest activity based on the chemical composition in %w/v while Jik showed the highest activity. The antibiotic susceptibility test showed that the isolate had multiple resistance by resisting seven out of the ten antibiotic used. The results were compared with in use dilution as recommended by the manufacturer of disinfectants. The three disinfectants are effective if used as directed with Jik as the most effective at 0.5v/w% MIC.

Keywords: Antibiotics, Clinical, Composition, Disinfectant, Inhibitory.

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INTRODUCTION

Hospital environments are highly contaminated with numerous pathogenic bacteria. In order to reduce transmission of these pathogens to patients, visitors and staff, disinfectants are used in making the environment safe. Disinfection in hospital practice is mainly achieved either by surface disinfection (e.g. disinfection of surfaces of tables, trolleys, instruments, walls and floors) or immersing the contaminated objects in the disinfectant solutions of certain dilution (Akabueeze *et al.*, 2013). However, the effectiveness of disinfectants is concentration dependent (Okesola and Olola, 2011).

The formulation of disinfectants, level of organic charge, synergy, temperature and dilution rate influence the antimicrobial activity of disinfectants (Russel, 2003). The mechanisms of action of disinfectants on bacteria include lysis and leakage of intracellular constituents (Christopher *et al.,* 2007), inhibition of enzymes, electron transport and oxidative phosphorylation (Mc Donnell and Russel, 1999) and effect on membranes (Denyer and Stewart, 1998).

Pseudomonas aeruginosa belong to a vast genus of aerobic, non-fermenting, saprophytic, Gram-negative bacilli widespread in nature particularly in moist environments (Ndip *et al.,* 2005). *Pseudomonas aeruginosa,* one of the most common organism found in hospital environment, this might be related to its ability to resist antibacterial, disinfectants and its ability to grow in moist conditions with simple nutrients (Davane *et al.,* 2014). *Pseudomonas aeruginosa* is commonly found in various places of hospital environment including the sinks, drains, taps, food, water, pharmacy preparations, contaminated hospital environment, mattresses and cleaning materials (mops and brushes) (Davane *et al.,* 2014). *Pseudomonas aeruginosa* is extensively studied for its high incidence and extraordinary potential to form biofilms. (Hill *et al.,* 2010). It is therefore imperative to investigate the effect of various disinfectants on the viability of *P. aeruginosa* from hospital environment.

Pseudomonas aeruginosa can grow on moist surfaces with simple nutrient, can resist antibacterial agent as well as disinfectants, for these reasons, the organism can and are easily found in various places of health care environment which include sinks, drains taps and other contaminated hospital equipment and materials. To a very great extent, *P. aeruginosa* is a very significant contaminant of pharmaceuticals and cosmetics. When present in pharmaceutical products it causes damage to the user and also caused inactivation of such medication. Basically, continuous and careful monitoring of the objects and sites *that P. aeruginosa* are isolated is necessary for the control of infection in the hospital environment, in patients and visitors. Regular environmental control measure can help control this type of hospital acquired infection (Lycsak *et al*., 2000; Gajadhar *et al*., 2003).

Antiseptics and disinfectants are used extensively in hospitals and other health care settings for a variety of topical and hard-surface applications. In particular, they are an essential part of infection control practices and aid in the prevention of nosocomial infections (Denyar *et al*., 1986; Eklund and Nes, 1991). Mounting concerns over the potential for microbial contamination and infection risks in the food and general consumer markets have also led to increased use of antiseptics and disinfectants by the general public. A wide variety of active chemical agents (or "biocides") are found in these products, many of which have been used for hundreds of years for antisepsis, disinfection, and preservation (Denyer *et al*., 1993). Despite this, less is known about the mode of action of these active agents than about antibiotics.

In general, biocides have a broader spectrum of activity than antibiotics, and, while antibiotics tend to have specific intracellular targets, biocides may have multiple targets. The widespread use of antiseptic and disinfectant products has prompted some speculation on the development of microbial resistance, in particular cross resistance to antibiotics. It is important to note that many of these biocides may be used singly or in combination in a variety of products which vary considerably in activity against microorganisms. Antimicrobial activity can be influenced by many factors such as formulation effects, presence of an organic load, synergy, temperature, dilution, and test method (Denyer *et al*., 1985, Russel *et al*., 1987, Block, 1991).

It is a known fact that the hospital environments are highly contaminated with numerous pathogenic microbes. The fact that hospital acquired infections is caused by microbes which are prevalent in hospital environment is known since long (Davane *et al.,* 2014).

Disinfections in hospital practice is mainly achieved either by surface disinfection (e.g. disinfection of surfaces of tables, trolleys, instruments, walls and floors) or immersing the contaminated objects in the disinfectant solutions of certain dilution (Akabueeze *et al.,* 2013).

Okesola and Olola (2011) showed that activity of disinfectants is concentration dependent *Pseudomonas aeruginosa,* because of its ability to grow in moist conditions with simple nutrients and because of its ability to resist antibacterial agents and disinfectants is commonly found in various places of hospital environment including the sinks, drains, taps,

food, water, pharmacy preparations, contaminated hospital environment, mattresses and cleaning materials (mops and brushes) (Davane *et al.*, 2014)

This work was carried out to evaluate the effectiveness of three (3) commonly used disinfectants in the hospitals in Nigeria on a clinical isolate of *Pseudomonas aeruginosa.*

MATERIALS AND METHODS

Selections and Sources of Disinfectants

The disinfectants used in this study were Dettol (chloroxylenon), Jik (sodium hypochlorite), and Izal (phenolic compound). These disinfectants are the commonly used disinfectants in hospitals in Nigeria. They were obtained from retail shops within the vicinity of Ondo State Trauma and Surgical Center, Laje road, Ondo, Ondo State, Nigeria.

Source of Clinical Isolate of *Pseudomonas Aeruginosa*

Clinical isolate was collected from the Laboratory of Ondo State Trauma and Surgical Center, Laje Road, Ondo, Ondo State, Nigeria.

Procedure for Mcfarland Standard

The McFarland standard (0.5) was prepared by adding 5ml of 1.175% Barium chloride dehydrate ($BaCl_2.2H_2O$), with 995ml of 1% Sulfuric acid (H_2SO_4). This is approximately about $1x10^8$CFU/ml of bacterial cell. The 0.5 McFarland standard was introduced into a curvet and the absorbance was measured in a spectrophotometer at 450nm (WPA Linton Cambridge UK Type S104D No 254) (Leboffe and Pierce, 2010).

Identification of Isolate

The isolate was identified based on the cultural appearance on Nutrient agar medium, the pigmentations, morphological arrangement using conventional microbiological techniques and biochemical tests according to Olutiola *et al*., 2000. The tests include gram staining, catalase, indole, citrate, methyl red, urease and Voges-Proskauer tests.

Preparation and Standardization of Inoculum

Five colonies were touched with a loop and growth transfer to 9mls of nutrient broth. The broth was inoculated at 35-37^{o}C until the growth reaches a turbidity equal or greater than that of 0.5 McFarland standard as described by ESCMID (2003). The culture was adjusted with sterile distilled water to give equivalent to the McFarland 0.5 standard. This was done photometrically (using 450nm and 1-cm path absorbance will be 0.08-0.10) (ESCMID, 2003).

Antibiotic Susceptibility

In vitro susceptibility of the identified *P.aureginosa* isolates against antimicrobial agents was determined by the standard disk diffusion procedure. The organisms were standardized using McFarland standard at the absorbance of 450nm. The samples were inoculated on Muller-Hinton agar. The following antimicrobial agents were tested: Septrin (SPT 30µg), Gentamicin (GEN 10µg), Ciprofloxacin (CPR 5µg), Perfloxacin (PFL 5µg), Ampicillin (AMP 10µg), Ampiclox (AMP 30µg), Erythromycin (E 10µg), Streptomycin (S 10µg), Zinnacef (Z 10µg) and Rocephin (R 10µg). Following the application of antimicrobial discs, the plates were incubated at 37°C for 24 h in an incubator (Royalcare England. DNP 9022A). The diameters of the zones of inhibition were measured (millimetres) and were compared to internationally accepted standard to determine the susceptibility or resistance of the isolate (Quinn *et al*., 1994).

Preparation of Dilution of Disinfectants

The serial concentration dilution of each of the disinfectants was done using sterile distilled water base on w/v or v/v of each of the disinfectants. The three disinfectants used are Jik (Sodium Hypochlorite) 3.5% w/v, Dettol (Chloroxylenol) 4.8% w/v and Izal (Phenolic compound) 35% w/v.

Preparation of Serial Dilution for Jik (Sodium Hypochlorite)

The serial dilution was done using sterile distilled water to obtain serial concentration of 0.1, 0.2%, 0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 3.5% w/v. To obtain these concentrations, 10mls of the stock was added to each of 470mls, 165mls, 60mls, 25mls, 13.3mls, 7.5mls and 4.0mls of water. For stock of 3.5% v/w no water is requires.

Preparation of Serial Dilution for Dettol (Chloroxylenol)

The serial dilution was done using sterile distilled water to obtain serial concentration of

0.125%, 0.25%, 0.5%, 1.0%, 1.5%, 2.0% 2.5%, 3.5% and 4.8%w/v. To obtain these concentrations, 10mls of stock was added to each of 375mls, 182mls, 86mls, 38mls, 22mls, 14mls, 9.2mls and 4.0 of water. For the stock of 4.8% w/v no water will added

Preparation of Serial Dilution for Izal (Phenolic Compound)

The serial dilution was done using sterile distilled water to obtain serial concentration of 0.25%, 0.5%, 1%, 5%, 10%, 15%, 20%, 25% and 35% w/v. To obtain these concentrations, 10mls of the stock was added to each of 1390mls, 690mls, 340mls, 60mls, 25mls, 13.33mls, 7.5mls and 4.0mls of water.

Inoculation and Incubation

Ten 10ml test tubes were used for inoculation and incubation. To 8ml of each nutrient broth prepared, 1 ml of standardized inoculum was introduced and 1 ml of various dilution of each disinfectant was introduced and incubated at 37^{o}C for 24 hours. Control experiment (Positive and Negative) were also be set up. After incubation, the series of the test tubes were observed for microbial growth, which is indicated by the turbidity. The last tube in dilution series that did not demonstrate growth corresponds with the minimum inhibitory concentration (MIC) of the disinfectants. Minimum inhibitory concentration (MIC). ESCMID (2003) was adopted.

RESULTS

The colony of isolate used in this research was flat with smooth edge and showed a green pigmentation on nutrient agar and the chemical result shown in Table1 identified the isolate as *Pseudomonas aureginosa*. The antibiotic susceptibility test showed that the *Pseudomonas aeruginosa* used in this research had multiple resistance. It showed resistance to 7 out of the 10 antibiotics used (Table 2).

Table 1: Confirmatory tests for identification of *Pseudomonas aureginosa*.

TEST	
Pigment	+
Motility	+
Gram staining	-
Catalase	+
Oxidase	+
Methyl Red (MR)	-
Indole	-
Citrate	+
Urease	-
Voges –Proskauer (VP)	-

Key + Positive, - Negative

Table 2: Antibiotic resistance strain of *Pseudomonas aeruginosa* clinical isolate from Diagnostic laboratory of Ondo State surgical and Trauma Center, Ondo

Antibiotics	Result (mm)	Interpretation
Zinnacef	14	R
Septrin	13	R
Streptomycin	27	S
Gentamycin	28	S
Ampicilin	15	R
Ampiclox	17	R
Ciprofloxacin	32	S
Perfloxacin	11	R
Erythromycin	18	R
Rocephin	11	R

R=resistant, I= intermidate, S=susceptible

Turbidity was observed in some of the test tubes after 24 hour of inoculation. The control in which no organism was added showed no turbidity after 24 hour of incubation, it was clear just as when the broth was prepared while the standard in which the organism was

introduced without addition of any disinfectant showed a great turbidity. Table 3 showed the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of Jik disinfectant used in this research. The minimum inhibitory concentration correspond to 0.5%w/v of the Jik disinfectant while the MBC of the Jik disinfectant was 1.0%w/v concentration. The MIC and MBC of Dettol corresponds to 1.5 and 2.0%w/v respectively (Table 4). Table 5 showed the turbidity trend of Izal disinfectant used in this research. It was observed that the MIC was 5% with the MBC of the disinfectant being 10.0%. The lowest activity of the three disinfectant based on the chemical composition in w/v was Izal while Jik showed the highest activity by percentage composition of the chemical content.

The results were compared with in use dilution as recommended by the manufactures on the label of each disinfectants. For Jik, the in-use dilution recommended is 0.6% w/v and the growth of *P. aeruginosa* is inhibited at 0.5% w/v. For Dettol, the in-use dilution recommended is 1.82% w/v and the growth of *P. aeruginosa* is inhibited at 1.5% w/v. For Izal, the in-use dilution recommended is 7.8% w/v and the growth of *P. aeruginosa* is inhibited at 10.0% w/v.

Table 3: The minimum inhibitory and bactericidal concentration of Jik (Sodium Hypochlorite) on *Pseudomonas aeruginosa* Isolate.

Concentration (%w/v)	0.1	0.2	0.5	1.0	1.5	2.0	2.5	3.5	In use dilution
MIC	Turbid	Turbid	Clear	Clear	Clear	Clear	Clear	Clear	
MBC	Growth	Growth	Growth	No-Growth	No-Growth	No-Growth	No-Growth	No-Growth	0.6

Table 4: The minimum inhibitory and bactericidal concentration of Dettol on isolate of *Pseudomonas aeruginosa*

Concentration (%w/v)	0.125	0.25	0.5	1.0	1.5	2.0	2.5	3.5	3.8	In use dilution
MIC	Turbid	Turbid	Turbid	Turbid	Clear	Clear	Clear	Clear	Clear	
MBC	Growth	Growth	Growth	Growth	Growth	No-Growth	No-Growth	No-Growth	No-Growth	1.82

Table 5: The minimum inhibitory and bactericidal concentration of Izal on *Pseudomonas aeruginosa* isolate

Concentration (%w/v)	0.25	0.5	1.0	5.0	10.0	20.0	25.0	35.0	In use dilution
MIC	Turbid	Turbid	Turbid	Clear	Clear	Clear	Clear	Clear	
MBC	Growth	Growth	Growth	Growth	No-Growth	No-Growth	No-Growth	No-Growth	7.86

Table 6: The MIC, MCB and In- use dilution result for the 3 Disinfectants

TEST	JIK	IZAL	DETTOL
MIC	**0.5**	**5.0**	**1.5**
MCB	**1.0**	**10.0**	**2.0**
IN-USE DILUTION	**0.6**	**7.86**	**1.82**

DISCUSSION

This study has shown that the activity of the disinfectants used in the hospital depends on the concentration of the disinfectant making contact with the organism involved as reported by previous researchers (Awodele *et al*., 2007; El-Mahmood and Doughari, 2009; Okesola and Olola, 2011). In this study, *Pseudomonas aeruginosa* was inhibited based on the concentration of the chemical composition of each of the tested disinfectant, this further confirm the claim that the activity of disinfectants are concentration based.

Jik inhibits the growth at concentration of 0.5%w/v has lethal effect at higher concentration of 1.0 %w/v. Jik is effect in inhibiting the growth of *Psuedomonas aeruginosa* at a concentration that is minimally low showing that it is effective and it is concentration based in as reported by Awodele *et al.*, 2007. Dettol inhibits the growth at concentration of 1.5%w/v and showed lethal effect at a higher concentration of 2.0%w/v. The concentration at which Dettol inhibits and

showed lethal effect indicated that Dettol is effective in controlling the growth of *Pseudomonas aeruginosa* not resistace to Dettol. Izal also inhibited growth and have lethal effect at 5%v/w and 10%w/v respectively in conformity with Alabi and Sanusi, 2012.

It is a known fact that *Pseudomonas aeruginosa* is a contaminant of the hospital environment and also implicated in outbreak of nosocomial infection especially in the intensive care units of the health facilities (Jones *et al*., 2003). Based on the ubiquity *P. aeruginosa* had developed resistance to some of the disinfectants that are used in hospital environment. This might be responsible for the high concentration of the chemical composition of the disinfectant required to overwhelm the bacteria. As reported by Health *et al*. (2001), some disinfectants has been reported to share the same mechanism of action with some antibiotics which can cause resistance to disinfectants used in cleaning our environment. It has been reported that *Pseudomonas aeruginosa* are with multiple resistance to antibiotics (Okesola and Olola, 2011). It can therefore be establishedthat the resistant to disinfectants especially in hospital environment could be antibiotic-resistant related as a result of cross-resistance. There may likely have been some molecular linkage in the resistant to disinfectant, Kaulfer *et al.,* (1987) referred to the resistance to disinfectant as a result of mutation and or presence of plasmid.

In a research carried out by El-Mahmood and Doughari (2009), it was reported that some of the disinfectants that are used in the hospitals in controlling nosocomial infection have been compromised in term of the chemical composition of such disinfectant. However, researches claim that most antimicrobial agent showed both inhibitory and lethal effect based on the concentration used and other factors such as degree of contamination and duration of treatment (El-Mahmood and Doughari, 2009). The three disinfectants used in this study showed both inhibitory and lethal effect as the concentration increases which conform with the report of El-Mahmood and Doughari, 2009.

The minimum inhibitory concentration is a parameter used in determining the bacteriostatic effect of a given disinfectant while the minimum bactericidal concentration is used to determine the bactericidal effect of the antimicrobial agent under the same condition. In this study, the MIC of Jik (Sodium hypochlorite) is 0.5 while the MCB is 1.0, the MIC of Izal is 5.0 and MCB is 10 while the MIC of Dettol is 1.5 and its MCB is 2.0.

The MIC is lesser than the MBC in all the three disinfectants used, this result correlate with the report of Ashley (1983) who studied the effect of two mouth washes against some buccal organisms and reported that the MIC is lower than the MBC. This is because the concentration at which the organisms would be killed is higher than the concentration at which the growth is inhibited (El-Mahmood and Doughari, 2009).

The antibiotic susceptibility test carried out on the organisms showed that the isolate of *Pseudomonas aeruginosa* used in this research showed a multiple resistance by being susceptible to only three out of the ten antibiotic used against it. The multiple resistance of *Pseudomonas aeruginosa* has been recorded by several researchers in which this study corresponds **(**Awodele *et al*., 2007; Prasanthi, *et al.,* 2012). Higgins *et al.* (2001) reported that *Pseudomonas aeruginosa* particularly demonstrate resistance to biocides which is the basic reason why the organisms are found having multiple resistance when treated with antibiotics and other antimicrobial agent.

The recommended concentration of manufacturers for Jik, Izal and Dettol for disinfection is 0.6, 7.8 and 1.82 respectively. The MIC for Jik, Izal and Dettol in this study are 0.5, 5.0 and 1.5 respectively. The MCB for Jik, Izal and Dettol in this study are 1.0, 10.0 and 2.0 respectively. The recommended concentration of the manufactures of the three disinfectants is higher than the result obtained indicating that the recommended concentrations of the manufacturers have inhibitory effect. Using lower concentrations of disinfectants than advised by the manufacturers will not inhibitory effect on the *Pseudomonas aeruginosa* and this confirmed the work of Majid *et al.,* 2013. The recommended concentrations of the manufacturers nevertheless do not have lethal effect as observed in the result.

These results pointed that treatment of *Pseudomonas aeruginosa* with sub-MIC of Claradone and Sarttol make this bacterium to become resistant to some antibiotics. These results were consistent with those of Olukemi and Funmilayo (2011), who found that the use of sub-inhibitory concentrations of the disinfectants causes a development in resistance and virulence of bacterial strains. They concluded that using lower concentrations

of disinfectants than advised by the manufacturers might have serious influence on bedridden patients.

In conclusion, this study showed that some of the serial concentrations have sub-optimal concentration. The active ingredient present in the three disinfectants used provided active at differing concentration. The activity of Jik was found to be the best in term of the concentration of active ingredients with 0.5%w/v MIC. This is an indication that *Pseudomonas aeruginosa* can be inhibited, which is an indication that Jik of this concentration can be used in the clinical setting for disinfection. The use of sub-optimal concentration might lead to the development of resistance and virulence strain of the organism. The use of concentrations of disinfectants lower than that observed in this research might have serious consequences in the management of nosocomial infection.

This study also emphasis the need for hospitals to have standard disinfection policy and adhere strictly to it. This will go a long way to curb the dissemination or transmission of resistant strain and nosocomial infection in our health care facilities.

It should be noted that all the disinfectants are effective against the isolate of *Pseudomonas aeruginosa*, but at different concentration. It is therefore imperative to increase the concentration of the disinfectants in order to get a very good result.

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APPENDIX

Antibiotic standard

Antibiotic	Susceptibility	Intimidate	Resistance
Septrin	≥19	16-18	≤15
Gentamicin	≥26	23-25	≤22
Ciprofloxacin	≥30	27-29	≤26
Perfloxacin	≥18	16-17	≤15
Ampicillin	≥19	16-18	≤15
Ampiclox	≥28	22-27	≤21
Erythromycin	≥26	23-25	≤22
Streptomycin	≥26	23-25	≤22
Zinnacef	≥20	17-19	≤16
Rocephin	≥24	21-23	≤20

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