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Evaluation of Bactericidal and Bacterostatic Effect of Commercially Produced Disinfectants in Awka Metropolis, Nigeria

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Abstract:

The most in practice is the chemical method by using chemicals called disinfectants and these can be obtaining commercially. Some varieties of chemical disinfectants are available for killing some microorganism or at least preventing their multiplication, as such, this study was conducted on the evaluation of the bactericidal and Bacterostatic effect of some commercially produced disinfectants. Three disinfectants were used; Dettol (Reckitt Benkiser Pharmaceuticals Ltd), Trichlorophenol TCP (Neimeth int'l pharmaceuticals Plc) and Izal (Medreich PLC) on two bacterial isolates were studied. The test organisms used include Escherichia coli and Staphylococcus aureus. The various dilutions at which the disinfectants had bactericidal effect on the organisms were; Dettol (10-1: 0.163), TCP (10-1: 0.098, 10-2: 0.262, 10-3: 0.283) for E. coli. Dettol (10-1: 0.123, 10-2: 0.448), TCP (10-1: 0.002, 10-2: 0.004) for Staph. Aureus. But Izal exhibited Bacterostatic effect at dilutions; E. coli (10-1, 10-2, 10-3) showing 10-1: 1.4981, 10-2:1.557, and 10-3:1.686, while Staph. Aureus showed at 10-1:1.980, 10-2:1.998, and 10-3:1.998 respectively. It was observed that Dettol showed the highest bactericidal effect on the graving cells of E. coli and Staph. Aureus. Therefore, further work can be carried out on other available disinfectants with other resistant test organisms within the same study area for more information.

Keywords:

Bactericidal and Bacterostatic, Commercial Disinfectants, Effect on Pathogens, Evaluation, Test Organisms

1. Introduction

General hygiene and hygiene practices are aimed at controlling diseases and its infections (antiseptic) through either the physico – chemical and biological methods of controlling these diseases, in our home (domestically or personal practice) or industries, which are mostly caused by the diseases causing organisms known as pathogenic organisms such as bacteria, protozoan's, parasites, etc. The most in practice is the chemical method by using chemicals called disinfectants and these can be obtaining commercially.

A variety of chemical disinfectants are available for killing bacteria or at least preventing their multiplication [1, 2]. These disinfectants are either bactericidal or bactericidal [3]. Factors like concentrations of disinfectants, time of exposure, temperature of disinfections; numbers of microorganisms present and the nature of materials being disinfected determine the action of disinfectants [4, 5]. Disinfectant, an agent that will destroy many of the disease causing microorganism present on the surface of an inanimate object, from a technical sense [6, 7]. These compounds (chemical disinfectant) after possible chemical manipulation provide and improved drugs to treat the infectious diseases by destruction of causative agent [8].

Bactericidal irreversibly inactive essential cells function of the organism, there are chemical disinfectants that prevent multiplication of the target bacteria only when in contact with the microorganism [9, 10]. Removal of these agents from the target bacterial leads to the resumption of microbial growth when the bacteria are placed in a suitable environment these chemicals are known as bacteriostatic chemical disinfectants are toxic to or other target organs like cell wall and cell membrane of the organisms that is why they are not suitable as chemotherapeutic agent [9, 10, 11, 12]. A disinfectant must be capable of reducing the level of pathogenic bacteria by 99.99% during a time frame greater than 5 less than 10 minutes [7, 11]. Such products, the antimicrobial activities should be tested and examined against some known pathogenic microbes in order to confirm these activities associated and ascertain the parameters associated with it [13].

Applications of some chemicals commonly known as disinfectants, this is in order to control and prevent infections due to microorganisms, are the part of hygiene practice, and these disinfectants mostly are obtainable commercially. Sometimes, these chemical disinfectants fails to be effective and the organisms (pathogenic organisms or diseases causing organisms) that are targeted became resistance due to lack of the appropriate formulation and the application methods. The objective of the research study is aimed at the determination of effect of some commercially available disinfectants on the inhibitory or destructive effect to specific bacteria.

2. Materials and Methods

2.1. Materials

Various chemicals, instrument, media and reagents used in this study were of highest analytical grade available and were obtained from the Biotechnology

Research Centre, Nnamdi Azikiwe University, Awka, Nigeria. The research was conducted at General laboratory of the Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University Awka, and SOP was highly observed.

2.2. Standard Test Organisms

The standard test organisms used for this study were *Escherichia Coli* (BN. 224) and *Staphylococcus aureus* (BN. 225) which were obtained from Clanson Laboratory, Awka, Nigeria. While the standard disinfectants used in the study were obtained from Awka main market and these were Dettol manufactured byReckitt Benkiser Pharmaceuticals Ltd, Trichlorophenol – (TCP) manufactured by Neimeth International Pharmaceuticals PLC and IZAL manufactured by Medreich PLC respectively.

2.3. Methods

2.3.1. Isolation Media and Inoculum

Procedure: - The isolation media used for this study was nutrient agar (NA) and the inoculums (test organisms) were *Escherichia coli* and *Staphylococcus aureus*. The required components were dissolved in 500 ml of distilled water, followed by autoclaving at 121^{o}C for 15 minutes. The medium was removed, allowed to cool to temperature of about 56 ^{o}c and then poured in to plates, allowed to solidify. The inoculums were inoculated each on poured plates and incubated at 37 ^{o}c for 24 hours.

2. 3.2. Determination of Microbial Growth

Procedure: The growth of each test organisms; *Escherichia coli* and *Staphylococcus aureus* were routinely subculture on peptone water in a bijou bottle each, and then the test bacteria (Inoculums) were inoculated on Petri dishes contained the medium and incubated at 37 ^{o}c for 24 hours.

2.3.3. Assay of Disinfectant Activity

Procedure: 100 % (v / v) disinfectant was prepared for each test tube by adding 1 ml of test bacterium in to 9 ml of different disinfectants (Dettol, Izal and Trichlorophenol). The disinfectant and the test bacteria were serially diluted into the test tubes. 1 ml of the inoculums broth were subsequently transferred aseptically in to the various test tubes in a serial of ten - fold dilutions (10^{-1}, 10^{-2}, and 10^{-3}), using a sterile different pipette, aseptically transfer 1 ml of inoculums to the 1st tubes of each column (1 / 10), shaken properly to mix and were incubated at 37 ^{o}c for 18 – 24 hours. That is 10^{-1}, the same was repeated for the other two columns and mixed thoroughly to obtain 10^{-2}, 10^{-3}dilutions, and then the last 1 ml was discarded. Aseptically inoculated 0.02 ml of the diluted sample into the plates, and then spread on plates. The plates were incubated at 37 ^{o}c for 18 - 24 hours. That is 10^{-1}, the same was repeated for other two columns and mixed thoroughly to obtain 10^{-2} and 10^{-3} dilutions, and then the last 1 ml was discarded. Aseptically inoculated 0.02ml of the diluted sample into the plates, and then spread on plates. The plates were incubated at 37 ^{o}c for 18 - 24hours.

2.3.4. Standard Plate Count of Bacterial Growth in Disinfectants

Procedure: - Bacterial standard plate count is counting the number of viable cells present after dilution of bacterial into disinfectants on a nutrient agar plate. The 0.05 ml dilution was pipette onto the prepared plate as labeled 10^{-1}, 10^{-2}, and 10^{-3} with a control set up, the plates were streaked out and incubated at 37oc for 24 hours (Quebee 205 USA) counter was used for the estimation of viable cells count.

2.3.5. Estimation of Bacterial Growth Using Spectrophotometer

Procedure: - 0.5 ml of each disinfectant and bacterial culture were dispensed into the cuvette, and light waves were allowed to pass through the samples at a wavelength of 600 nm. The optical readings (OD) were obtained for each sample.

3. Results and Discussion

Some chemical substances have active ingredients that capable destroying (Lysol) some microbes if not all, in form of bactericidal or bacteriostatic activity. These chemical substances are call disinfectants and the process is term disinfections. This is in order to control and prevent infections due to microorganisms, are the part of hygiene practice, and these disinfectants mostly are obtainable commercially. Sometimes, these chemical disinfectants fails to be effective and the organisms (pathogenic organisms or diseases causing organisms) that are targeted became resistance due to lack of the knowledge of appropriate formulation and the application methods.

As a result of the statement made above, this necessitate the research study determined and ascertained the effect of some commercially available disinfectants (Dettol, Izal and TCP) on the inhibitory or destructive effect to specific bacteria (*E. coli* and *Staph. aureus*) and results obtained were reported and presented on the tables below as follows:

3.1. Results

Table 1 showed the plate count of the test organism; *E. coli* in serial dilutions (10^{-1}, 10^{-2} and 10^{-3}) of the disinfectants (Dettol, Izal and TCP) with it control of 1 ml dilution yielded a viable count of 40 x 10^9 numbers of cells per ml (n / ml). Dettol at the dilution of 10^{-1}had no growth, 10^{-2} had 2 x 10^9 n / ml and 10^{-3} had 3 x 10^9 n / ml. Izal at the dilution of 10^{-1} had 10 x 10^9 n / ml, 10^{-2} had 12 x 10^9 n / ml and 10^{-3} had 15 x 10^9 n / ml. While TCP at the dilution of 10^{-1} had 10 x 10^9 n / ml, 10^{-2} had 15 x 10^9 n / ml and 10^{-3} had 17 x 10^9 n / ml respectively.

Table 1. *Plate Count of E. coli Cultured on Different Dilutions of the Disinfectants.*

Disinfectant	**Dilution Factor**	**Colony Estimation (n / ml)**
Control	1 ml	40
Dettol	10^{-1}	No growth
	10^{-2}	2
	10^{-3}	3
Izal	10^{-1}	10
	10^{-2}	12
	10^{-3}	15
TCP	10^{-1}	10
	10^{-2}	15
	10^{-3}	17

Keys: n / ml = number of cells per millitres

Table 2 showed the result of the plate count of the test organism; *Staph. aureus* in serial dilutions (10^{-1}, 10^{-2} and 10^{-3}) of the disinfectants (Dettol, Izal and TCP) with it control of 1 ml dilution yielded a viable count of 40 x 10^{9} numbers of cells per ml (n / ml). Dettol at the dilution of 10^{-1} had no growth, 10^{-2} had 3 x 10^{9} n / ml and 10^{-3} had 4 x 10^{9} n / ml. Izal at the dilution of 10^{-1} had 10 x 10^{9} n / ml, 10^{-2} had 20 x 10^{9} n / ml and 10^{-3} had 24 x 10^{9} n / ml. While TCP at the dilution of 10^{-1} had 6 x 10^{9} n / ml, 10^{-2} had 9 x 10^{9} n / ml and 10^{-3}had 12 x 10^{9} n / ml.

Table 2. *Plate Count of Staph. aureus Cultured on Different Dilutions of the Disinfectants.*

Disinfectant	**Dilution Factor**	**Colony Estimation (n / ml)**
Control	1 ml	40
Dettol	10^{-1}	No growth
	10^{-2}	3
	10^{-3}	4
Izal	10^{-1}	10
	10^{-2}	20
	10^{-3}	24
TCP	10^{-1}	6
	10^{-2}	9
	10^{-3}	12

Keys: n / ml = number of cells per millitres

Table 3 showed the results of optical density of the disinfectants used in this study on the test organism; *E. coli* in serial dilutions (10^{-1}, 10^{-2} and 10^{-3}) of the disinfectants (Dettol, Izal and TCP) with it control of 1 ml dilution and with optical density of 0.618 at a wavelength of 600 nm. For the Dettol, at the dilution of 10^{-1} had an optical density of 0.123 nm, 10^{-2} had 0.445 nm and 10^{-3} had 0.674 nm. Izal at the dilution of 10^{-1} had optical density of 1.980 nm, 10^{-2} had 1.994 nm and 10^{-3} had 1.998 nm. Finally, TCP at the dilution of 10^{-1} had optical density of 0.002 nm, at 10^{-2} had 0.004 nm and 10^{-3} had 1.594 nm respectively.

Table 3. *Optical Density of Disinfectant on E. coli at Wave Length 600 nm.*

Disinfectant	**Dilution Factor**	**Colony Estimation (n / ml)**
Control	1 ml	0.618
Dettol	10^{-1}	0.123
	10^{-2}	0.445
	10^{-3}	0.674
Izal	10^{-1}	1.980
	10^{-2}	1.994
	10^{-3}	1.998
TCP	10^{-1}	0.002
	10^{-2}	0.004
	10^{-3}	1.594

Keys: nm = Nanometer

Table 4 showed the results of optical density of the disinfectants used in this study on the test organism; *Staph. aureus* in serial dilutions (10^{-1}, 10^{-2} and 10^{-3}) of the disinfectants (Dettol, Izal and TCP) with it control of 1 ml dilution and with optical

density of 0.744 at a wavelength of 600 nm. The Dettol, at the dilution of 10^{-1} had an optical density of 0. 163 nm, at 10^{-2} had 0.289 nm and 10^{-3} had 0.717 nm. For the Izal at the dilution of 10^{-1} had optical density of 1.198 nm, at 10^{-2} had 1.557 nm and 10^{-3} had 1.686 nm. Finally, TCP at the dilution of 10^{-1} had optical density of 0.098 nm, at 10^{-2} had 0.262 nm and 10^{-3} had 0.283 nm.

Table 4. *Optical Density of Disinfectant on Staph. aureus at Wave Length 600 nm.*

Disinfectant	**Dilution Factor**	**Colony Estimation (n / ml)**
Control	1 ml	0.744
Dettol	10^{-1}	0.163
	10^{-2}	0.289
	10^{-3}	0.717
Izal	10^{-1}	1.198
	10^{-2}	1.557
	10^{-3}	1.686
TCP	10^{-1}	0.098
	10^{-2}	0.262
	10^{-3}	0.283

Keys: nm = Nanometer

Table 5 showed the test organisms (*E. coli* and *Staph. aureus*) growth optical densities in different dilutions of disinfectants (Dettol, Izal and TCP). It revealed that *E. coli* in Dettol tube was observed in a scanty and moderate turbidity (turbidity) at 10^{-1} and 10^{-2}dilutions, while heavy turbidity was shown at 10^{-3} dilution. In Izal tubes, *E. coli* showed no physical turbidity in10^{-1}, 10^{-2} and 10^{-3} dilutions. It was observed that in TCP, *E. coli* showed heavy turbid appearance. *Staph. aureus* in Dettol tubes, it was observed that,there was no turbidity appearance in 10^{-1}, 10^{-2} and 10^{-3} dilutions at all and same with Izal tubes no turbidity was observed in 10^{-1}, 10^{-2} and 10^{-3} dilutions. *Staph. Aureus* in TCP tubes, scanty turbidity were observed in $10^{-1}$and 10^{-2} dilutions, while at 10^{-3} dilution, there was heavy turbidity being observed.

Table 5. *Test Organisms Growth in Different Dilutions of Disinfectants.*

Test Organism Used	**Disinfectant Used**	**Dilution Factors**	**Optical Density (n / m)**
E. coli	Dettol	10^{-1}	+ +
		10^{-2}	+ + +
		10^{-3}	++ + +
	Izal	10^{-1}	-
		10^{-2}	-
		10^{-3}	-
	TCP	10^{-1}	+
		10^{-2}	+ +
		10^{-3}	+ + + +
Staph. aureus	Dettol	10^{-1}	-
		10^{-2}	-
		10^{-3}	-
	Izal	10^{-1}	-
		10^{-2}	-
		10^{-3}	-
	TCP	10^{-1}	+ +
		10^{-2}	+ +

		10^{-3}	+ + + +

Keys: - = No Turbid, + = Less Turbid, + + = Scantily Turbid, + + + = Moderately Turbid, + + + + = Heavily Turbid.

3.2. Discussion

Hygiene and hygiene practices at our environment either in home or domestic or personal hygiene are aimed at controlling diseases and its infections through either the physico – chemical and biological methods of controlling these diseases, which are mostly caused by the diseases causing organisms known as pathogenic organisms such as bacteria, protozoan's, parasites, etc. The most in practice is the chemical method by using chemicals called disinfectants and these can be obtaining commercially. Applications of some chemicals commonly known as disinfectants, this is in order to control and prevent infections due to microorganisms, are the part of hygiene practice, and these disinfectants mostly are obtainable commercially. Sometimes, these chemical disinfectants fails to be effective and the organisms (pathogenic organisms or diseases causing organisms) that are targeted became resistance due to lack of the appropriate formulation and the application methods. Research study was carried out and determined the effects of some commercially available disinfectants (Dettol, Izal and Trichlorophenol) on the inhibitory (Bacterostatic) or destructive (bactericidal) effect to some specific bacteria; *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*Staph. aureus*). According to Bassey *et al*, [14] who stated that some chemical agents have higher constituents (active ingredients) that are responsible for the destruction of pathogenic microorganisms, these are either bactericidal or bacteriostatic chemical agents called the disinfectants. Some of these chemical agents may possessed either higher or lower minimum bactericidal concentration and minimum inhibitory concentration. Also Mahapatra *et al*, [8] stated these compounds (chemical disinfectant) after possible chemical manipulation provide and improved drugs to treat the infectious diseases by destruction of causative agent.

In these findings, the results revealed that the dilutions factor of 10^{-1} for both *Staph. aureus* and *E. coli* indicate a bactericidal effect. As observed, the optical density of Dettol on *E. coli* at 10^{-1} is 0.163 with a control of 0744 indicate bactericidal activity and also at 10^{-2} and 10^{-3} at 0.289 and 0.717 respectively. Izal in *E. coli* at 10^{-1}, 10^{-2}, and 10^{-3} there were bacteriostatic activity with an optical density of (1.498, 1.557 and 1.686) respectively. TCP in *E. coli* at 10^{-1} is 0.098 indicating bactericidal effect at 10^{-2} and 10^{-3} (0.262 and 0.283) respectively, bactericidal effect was observed, the optical density of Dettol in *Staph. aureus* at 10^{-1} is 0.123 with a control of 1.618. TCP in E. coli at 10^{-1} is 0.098 indicating bactericidal effect at 10^{-2} and 10^{-3} (0.262 and 0.283) respectively, bactericidal effect was observed. As observed, the optical density of Dettol in *Staph. aureus* at 10^{-1} is 0.123 with a control of 0618 indicating bactericidal effects and also at 10^{-2} with 0.448 but were Bacterostatic at 10-3 with 0.674. Izal in *Staph. aureus* at (10^{-1}, 10^{-2}, and 10^{-3}) with (1.980, 1.994, and 1.998) respectively indicate Bacterostatic effect). TCP in Staphylococcus aureus at 10^{-1} is 0.002 indicates a bactericidal effect, also observed at 10^{-2} dilution with optical density value of 0.004, Bacterostatic effect at 10^{-3} dilution with 1.594 optical density values.

The bactericidal and Bacterostatic effects of some disinfectants, Dettol, Izal and Trichlorophenol (TCIP) were studied. The effect of Dettol from the various observation on *E. coli* showed that Dettol has a killing effect at 0.163 (4ml) at dilution

1/100, and this is conformed with the finding of Hugbo and Nwankwo [2] who reported the bactericidal effect of Dettol on *E. coli* at 1 / 10 as 0.100 for *Staph. aureus*, and also the killing effect of Dettol at 1 / 10 is 0.123. The effect of Izal on *E. coli* showed that there were bacteriostatic effect at 10^{-1}, 10^{-2}, 10^{-3} dilutions with 1.498 to 1.684 (u / ml), and this differed from the experimental report of Nwanyanwu [6] who reported bactericidal effect at 1.755 (u / ml) for *Staph. aureus*, inhibitory effect at 10^{-1} to 10^{-3} dilutions with 1.980 to 1.998 (u / ml) this were not in line with the report of Nwanyanwu [6] who reported the killing effect at 10^{-1} as 1.755 (u / ml). The effect of Trichlorophenol (TCP) on *E. coli*, the results indicates that TCP had a bactericidal effect at 10^{-1} dilution at 0.098, and this finding, confirmed with the findings of Ogunniuyi and Kolawolo [7], for *Staph. aureus* the bactericidal effect at 10^{-1} is 0.002 (u / m) but bacteriostatic at 10^{-3} is 1.594 (u / m) this finding also is in conformity with the report of Ogunniuyi and Kolawolo [7]. The differences in the antibacterial activity of these disinfectant studied were due to the differences in their chemical composition that invariably affect the concentration at which they exhibit bactericidal effect. Disinfectants are generally employed for domestic, laboratory and health care services. The choice of good disinfectant depends on a number of factors such as Non - toxicity, non potential damage to instrument applied on, cost effect and wide degree of microbial bactericidal and bacteriostatic ability.

Therefore, this research on resistant strain reveals that disinfectants have their maximum effect at high dose of application mat which most microbes exhibit a bactericidal effect, hence, for effective thorough disinfections. Dettol could be recommended to be selected among the wide variety of commercially produced disinfectants after exhibiting bactericidal effects to the resistant strain of test organisms *E. coli* and Staph. aureus at high concentrations. Izal indicates a bacteriostatic effect with the invest efficacy in inhibiting the two resistant strain organisms studied. TCP exhibited bactericidal effect at high concentration but it is relatively Bacteriostatic at no concentration.

4. Conclusions

The important of disinfectants at homes and industries cannot be over stressed. The indispensable use of disinfectants to eliminate microbes, have been traced to long history of man. The problem man is facing is that, some of the disinfectants are not able to inhibit the growing microbes due to development of resistant by microbes to the disinfectant. The results obtained from this present research study that was conducted, it revealed that amongst the wide variety of commercially produced disinfectants, the three chosen (Dettol, Izal and TCP), used and tested on the two microbes (E. coli and Staph. aureus) after exhibiting the, Dettol could be recommended to be selected among the wide bactericidal effects to the resistant strain of test organisms *E. coli* and *Staph. aureus* at high concentrations, followed by TCP which exhibited bactericidal effect at high concentration but it is relatively bacteriostatic at no concentration and then Izal indicates a bacteriostatic effect with the invest efficacy in inhibiting the two resistant strain organisms studied.

The bactericidal and Bacterostatic effects of these disinfectants were studied, and effect of Dettol from the various observation on *E. coli* showed that it has a killing effect at 0.163 (4ml) at dilution 1/100, and this is conformed with the finding of Hugbo and Nwankwo [2] who reported the bactericidal effect of Dettol on *E. coli* at 1 / 10 as 0.100 for *Staph. aureus*, and also the killing effect at 1 / 10 is 0.123. The effect

of Izal on *E. coli* showed that there were bacteriostatic effect at 10^{-1}, 10^{-2}, 10^{-3} dilutions with 1.498 to 1.684 (u / ml), and this differed from the experimental report of Nwanyanwu [6] who reported bactericidal effect at 1.755 (u / ml) for *Staph. aureus*, inhibitory effect at 10^{-1} to 10^{-3} dilutions with 1.980 to 1.998 (u / ml). This was not in line with the report of Nwanyanwu [6] who reported the killing effect at 10^{-1} as 1.755 (u / ml). The effect of Trichlorophenol (TCP) on *E. coli*, the results indicates that TCP had a bactericidal effect at 10^{-1} dilution at 0.098, and this finding, confirmed with the findings of Ogunniuyi and Kolawolo [7], for *Staph.aureus* the bactericidal effect at 10^{-1} is 0.002 (u / m) but bacteriostatic at 10^{-3} is 1.594 (u / m) this finding also is in conformity with the report of Ogunniuyi and Kolawolo [7]. The differences in the antibacterial activity of these disinfectant studied were due to the differences in their chemical composition that invariably affect the concentration at which they exhibit bactericidal effect. Disinfectants are generally employed for domestic, laboratory and health care services. The choice of good disinfectant depends on a number of factors such as Non - toxicity, non potential damage to instrument applied on, cost effect and wide degree of microbial bactericidal and bacteriostatic ability. Therefore, the magnitude of effective and exhibiting power of anti-microbes in descending order was; Dettol > TCP > Izal disinfectant. The effective disinfections by disinfectants are strongly advocated. There is need to improve on the active ingredient that will enhance fast killing of the microbes and avoidance of being toxic to the host.

Recommendations

We recommend that more intensive study is to be carried out on resistant microbes, especially resistant Staphylococcus to various disinfectants. More research should be conducted in order to find a lasting solution to resistant strains of microorganisms to the disinfectants.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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