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Biological Evaluation of the Antibacterial efficiency of some Biodegradable Detergents and some Commercial Disinfectants

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Abstract

Antiseptics and disinfectants are used extensively in hospitals and other health care settings for a variety of topical and hard-surface applications. However, their antibacterial effectiveness is not always well declared by the manufacturers and consumers find it difficult to choose the right product according to their needs. Four biodegradable detergents: K3, NanoClear, NanotolPrimer and NanoFix, products of commercial company were tested for their antimicrobial activity and compared with commercial disinfectants such as Dettol and Clorox against Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27953, Bacillus cereus ATCC 33018, Staphylococcus aureus ATCC 29213. Minimum inhibitory concentration (MIC) and Lethal Contact Time was carried out. The results revealed that %10 Nanofix, %3 NanotolPrimer, %50 NanoClear except for P.aeruginosa was %100 and %10 K3 except for P.aeruginosa was %15, Dettol %5 and Clorox %5. The results showed that: Nanofix and Nanotol Primer had an effective antimicrobial effect nearly similar to that of Dettol and Clorox while K3 affected the tested microorganisms moderately and Nanoclear affected all tested bacterial pathogens only in the concentrated form without dilution. Also a test of Contact Lethal Time of concentrated detergents against test organisms at time intervals 0, 30 seconds and 1, 2, 5, 10 minutes was carried out and the results showed that the biodegradable detergents were acting as antiseptics not as disinfectants while the commercial disinfectants (Dettol and Clorox) were disinfectants showing no growth of bacterial cells after contact time less than 30 seconds.

Key words : Detergents , Disinfectants, Minimum inhibitory concentration (MIC) and Contact Lethal Time.

I. Introduction

Antiseptics and disinfectants are chemical compounds commonly added to water for use during bath, laundry, mouth washing, wound dressing and other domestic activities such as toilet and general house cleaning **(Akimitsu *et al.,* 1999)**. They are used to control or reduce the growth of pathogenic microbes found on human body **(Fraise, 2002).** Most of the antiseptics contain one of the following compounds: chlorhexidine, phenol, chloroxylenol and cetylpyridinium chloride (CPC) **(Giuliana *et al.,* 1997)**. All, with the exception of the mouthwash, are applied externally to prevent proliferation of microbial population particularly during bath.

But a common problem is the selection of disinfectants and antiseptics because different pathogens vary in their response to different antiseptics or disinfectants **(Russell 1996)**. Dettol is widely used in homes and healthcare settings for various purposes including disinfection of skin, objects and equipments, as well as environmental surfaces. With prior cleaning before application, the number of microorganisms colonizing the skin and surfaces are greatly reduced **(Rutala 1996)**. The antimicrobial properties of chloroxylenol, the main chemical constituent of Dettol and other chlorinated phenols have been extensively studied **(Hugo and Bloomfield 1971a)**. The antimicrobial properties of the disinfectant against some pathogenic bacteria have earlier been reported **(Mellefont**

***et al*. 2003).** Antimicrobial Efficacy of Selected Disinfectants cause distraction either by coagulating the protein of the bacteria, by destroying its cell membrane or by removal of a sulphonhydric group from the organisms **(Horton and Parker, 2002).** Also according to **Bernard (2003)** the mode of action of disinfectants is thought to be linked to destruction of proteins, lipids or nucleic acids in the cells or its cytoplasmic membrane, although microorganisms differ in their sensitivity to chemical germicide. The gram positive and gram negative microorganisms are implicated in different diseases and infections **(Johnson *et al.,* 2002)**. The content of many chemical agents can be expressed by more than one notation. In dilutions, a small volume of the liquid chemical (solute) is diluted in a large volume of solvent to achieve a certain ratio. For example, a common laboratory phenolic disinfectant such as Lysol is usually diluted 1:200 parts of water by volume. In general, solutions of low dilution or high percentage have more of the active chemical (are more concentrated) and tend to be more biocide, but expense and potential toxicity can necessitate using the minimum strength that is effective **(Lucet *et al*., 2002).**

The present study was aimed to determine the bactericidal activity/efficacy of both the disinfected and the detergent to determine, whether the detergent only removes the bacteria from skin or it also kills the bacteria. In the present study antimicrobial activity of commercial detergents and disinfected against *Escherichia coli*, *Pseudomonas aeruginosa, Bacillus cereus*, and *Staphylococcus aureus* were investigated.

II. Materials and Methods

Test Microorganisms and cell suspensions:

Gram negative bacteria: - *Escherichia coli ATCC 25922* , *Pseudomonas aeruginosa ATCC 27953*
Gram positive bacteria: - *Bacillus cereus ATCC 33018* ,*Staphylococcus aureus ATCC 29213* .
Growth Medium for Bacteria was Nutrient-Agar. Cell suspensions of 24h old bacteria, were made in saline solution pH.7 not less than 10^6 cells/ml.

Detergents and Commercial disinfectants:
Biodegradable detergents: K3 Floor Cleaning Agent , NanoClear multi-surface cleaning agent , Nanotol Primer cleaner and sealer and NanoFix (Bathroom Cleaner) All are products of commercial company. the active ingredient is alcohol ethoxylates.
Commercial disinfectants: Dettol was a product of Royal Cosmetic Co. Egypt under the licence of Reckitt Benckiser UK. The active ingredient is chloroxylonol and Chlorox was a product of Egyptian Company for Household Detergents, 10^{th} of Ramadan city, industrial district B2 with the authorization of CLOROX Company. The active ingredient is sodium hypochlorite.

Preparation of recommended concentration of the chemical disinfectants:

All concentrations were made in distilled H_2O

Minimum inhibitory concentration test (MIC)
The minimum inhibitory concentration is the highest dilution which fails to show growth. In this test, the required dilutions were prepared. The liquefied nutrient agar medium was inoculated by1 ml of the microbial culture suspension, poured in Petri- dishes and left to solidify. Wells of 1 cm diameter were made. Each well was inoculated with 0.1 ml of each of the sample dilutions separately. Dishes were incubated at 35°C for 48 h.
The inhibition zones produced if any were measured in cm.
Evaluation of the efficacy of chemical disinfectants:

Test of Lethal Contact Time:

Bacterial suspensions were prepared. Ten ml of the tested chemical disinfectant (stock solutions without dilution) were poured into sterile test tubes, 0.1 ml of the bacterial suspension was added and

shaken thoroughly to give the chance for micro-organism to come in contact with the disinfectant. At time intervals: 30 seconds, 1, 2, 5 and 10 min from original zero time, 1 ml of disinfectant-bacterial mixture were taken into a sterile test tube containing 9 ml of in-activator Tween 80 (1%) in nutrient broth, mixed thoroughly. One ml from in-activator mixture was inoculated in sterile Petri dish for each type of bacteria separately using pour plate method **(Cruickshank et al., 1980)**. Dishes were incubated at 35°C for 48 h

III. Results and discussion

The objective of this study was to evaluate the efficacy of some disinfectants which are proven to be biodegradable, environmentally safe and compare them with some commercially used chemical disinfectants.

The MIC test was carried out for each of the tested detergents separately against the bacterial pathogens, results showed that: all tested detergents and chemical disinfectants were effective against tested bacterial pathogens as stock solutions (100%). The inhibition zones measured after 48 h, when the 100% NanoFix was used were, 2.3 cm for *Staph.aureus* , 2.2 cm for *E.cloi* , 3.2 cm for *P.aeruginosa* and 2,2 cm for *B.cereus* (Table 1) while, the inhibition zones when 100% Dettol was used were 2.9, 3.2, 5.7 and 2.3 cm for *Staph aureus, E.coli, P.earuginosa* and *B. cereus* respectively.The measured inhibition zones when chlorox was used were 5.7, 6.2 5.9, 6.3 respectively (Tables 5). **EL Mahmood and Doughari (2008)** had reported that the efficacy of the liquid disinfectant Dettol against nosocomial *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* was investigated. Use dilutions of the disinfectant were not immediately lethal to the microorganisms, with the survival curves exhibiting an initial shoulder before exponential order of death.

Measuring the inhibition zones of tested bacterial pathogens when different concentrations of the product Nanofix were used showed that, the product had an antibacterial effect expressed by the inhibition zones of 1.4, 1.4, 1.5 cm for *Staph.aureus, E.coli* and *B.cereus* up till a dilution of %5 while *P.aeruginose* was not inhibited by this concentration. *P.aeruginosa* measured an inhibition zone of 1.4 cm at a concentration of %10 (Table 1).

Inhibition zones of 1.1, 1.5, 2.5 and 1.5 cm were measured for *Staph.aureus, E.coli, P.aeruginosa* and *B.cereus* respectively when the product NanotolPrimer was used at a concentration of %3. While inhibition zones measured 1.2, 1.2 and 1.4 cm for *Staph.aureus, E.coli* and *B.cereus* when a concentration of %50 of the product NanoClear was used. *P.aeruginosa* was affected by Nanoclear only in the concentrated form (Table 2).

The different concentrations of the product K3 resulted in inhibition zones of 1.2, 1.3 and 1.5 cm for *Staph.aureus, E.coli* and *B.cereus* at a concentration of %10 while, *P.aeruginosa* gave an inhibition zone of 1.3 cm when the concentration was %15 (Table 3).

Determination of MIC of Biodegradable Detergents:

Table (1): Determination of MIC of Nanofix against some pathogenic bacteria after 48 h.

IZ(cm)	Concentration of Nanofix (%)							
	100	**75**	**50**	**25**	**20**	**15**	**10**	**5**
Staph.aureus	2.3	2.3	1.9	1.9	1.5	1.4	1.4	0.0
E.coli	2.2	2.2	2.0	1.8	1.8	1.4	1.4	0.0
P.aeruginosa	3.2	3.1	3.0	2.0	1.8	1.4	0.0	0.0
B.cereus	2.2	1.8	1.7	2.4	1.7	1.5	1.5	0.0

IZ= inhibition zone

Table (2): Determination of MIC of Nanotol primer against some pathogenic bacteria after 48 h.

IZ(cm)	Concentration of Nanotol primer (%)								
	100	75	50	25	20	15	10	5	3
Staph.aureus	2.6	2.8	2.8	2.8	2.8	2.2	2.0	1.4	0.0
E.coli	2.7	2.5	2.0	4.0	3.5	3.4	3.4	1.6	1.5
P.aeruginosa	5.2	3.0	2.9	2.8	2.8	2.7	2.5	2.5	0.0
B.cereus	2.8	2.5	2.4	2.2	2.0	2.0	2.1	1.7	1.5

IZ= inhibition zone

Table (3): Determination of MIC of NanoClear primer against some pathogenic bacteria after 48 h.

IZ(cm)	Concentration of NanClear (%)			
	100	75	50	25
Staph.aureus	1.5	1.3	1.2	0.0
E.coli	1.7	1.4	1.2	0.0
P.aeruginosa	4.5	0.0	0.0	0.0
B.cereus	1.8	1.5	1.4	0.0

IZ= inhibition zone

Table (4): Determination of MIC of K3 against some pathogenic bacteria after 48 h.

IZ(cm)	Concentration of K3 (%)						
	100	75	50	25	20	15	10
Staph.aureus	2.5	2.5	2.1	1.5	1.3	1.3	0.0
E.coli	2.7	2.5	1.8	1.8	1.8	1.4	0.0
P.aeruginosa	3.5	3.0	2.4	1.7	1.3	1.3	0.0
B.cereus	2.8	2.8	2.2	2.0	1.6	1.3	1.3

IZ= inhibition zone

Determination of MIC of Commercial Disinfectants:

Table (5): Determination of MIC of Dettol against some pathogenic bacteria after 48 h.

IZ(cm)	Concentration of Dettol (%)								
	100	75	50	25	20	15	10	5	3
Staph.aureus	2.9	2.9	2.7	2.3	1.7	1.5	1.6	1.6	0
E.coli	3.2	3.5	3.0	2.9	2.8	2.8	2.6	2.4	2.3
P.aeruginosa	5.7	6.0	5.0	4.8	4.0	4.0	3.8	2.5	2.0
B.cereus	2.3	2.7	2.5	2.5	2.5	2.5	2.5	2.4	2.0

IZ= inhibition zone

Table (6): Determination of MIC of collorx against some pathogenic bacteria after 48 h.

IZ(cm)	Concentration of collorx (%)							
	100	75	50	25	20	10	5	3
Staph.aureus	5.9	5.9	5.7	5.3	3.7	3.6	2.6	2.5
E.coli	6.2	6.5	6.0	5.9	3.8	3.6	2.4	2.1
P.aeruginosa	5.7	6.0	6.0	5.8	4.0	3.8	2.5	1.8
B.cereus	6.3	6.7	6.5	6.5	4.5	3.5	2.4	1.8

IZ= inhibition zone

In the lethal contact time test, bacterial suspensions (>10^6) of each of the pathogens under test, came in contact with each of the concentrated detergents separately for time intervals of 30 seconds and 1, 2, 5, 10 mint then recultivated on respective media, bacterial growth was observed after the contact time intervals for all bacterial types in case of the biodegradable detergents under test. In case of contact time of Dettol or Clorox with bacterial pathogens under test, no growth was observed in time less than 30 second. **Okore *et al.* (2014)** had reported that the potency of disinfectants is very important to enhance the antimicrobial activity of these disinfectants towards controlling microbial population which includes prevention of diseases transmission and infection. It also prevents deterioration and spoiling of materials which could also lead to microbial infection. Determination of antimicrobial effectiveness of disinfectants is essential to achieve total disinfection of pathogens. The use of good disinfectants should be encouraged to reduce cases of skin and wound infections caused by most microorganisms.

Table (7): Determination of leathel contact time of biodegradable detergents against some pathogenic bacteria.

Time (min)	Nanofix					Nanotol					Nanocear					K3				
	0.5	1	2	5	10	0.5	1	2	5	10	0.5	1	2	5	10	0.5	1	2	5	10
Staph.aureus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E.coli	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P.aeruginosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B.cereus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Growth +

Table (8): Determination of leathel contact time of commercial disinfectants against some pathogenic bacteria.

Time (min)	Dettol					collorx				
	0.5	1	2	5	10	0.5	1	2	5	10
Staph.aureus	-	-	-	-	-	-	-	-	-	-
E.coli	-	-	-	-	-	-	-	-	-	-
P.aeruginosa	-	-	-	-	-	-	-	-	-	-
B.cereus	-	-	-	-	-	-	-	-	-	-

No growth –

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