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Study of Broad Spectrum Disinfectants Antibacterial Effect against Common Nosocomial Bacteria

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Antiseptics and disinfectants are extensively used in nosocomial systems and health care units and have important role in preventing the nosocomial infections. Resistance to these agents is very important and can cause serious problems dealing with multidrug resistant strains. The purpose of this study was to assess the *in vitro* activity of a group of disinfectants against nosocomial bacteria. Disc susceptibility testing is the most commonly recommended method for routine testing, and is described in the NCCLS 2004 standards. The Muller Hinton agar, Nutrient agar and Nutrient broth mediums and bacterial turbidity equal to 0.5 Mcfarland were used to assess the antimicrobial effect of Iodine, Cetrimide, Microtene %5, Formalin %37 and Deconex using disc diffusion method. The results show that Iodine had no or very low antimicrobial effect on gram negative pyogenic bacteria and no effect on acid fast and spore producing bacteria. Combination of Iodine and Cetrimide was fully synergistic against strains tested. The formalin 5% and Microten 37% had highest disc diffusion zone diameters and showed bactericidal activity on gram positive, gram negative, acid fast and sporulating bacteria. Although eradication of all germs in nosocomial systems is impossible, they can be controlled.

Key words: Bacteria, Nosocomial, Disinfectants, Antibacterial activity.

Nosocomial infections are one of the most problematic issues not only in the developing but also in the developed countries. Because of their important role in both prevention and control of nosocomial infections; antiseptics and disinfectants have been widely used in hospital and health care facilities[1]. Using antiseptics and disinfectants are increased drastically and pharmaceutical companies have perpetually been trying to encourage the widespread consumption of such products through advertising campaigns[2].

Nevertheless, little is known about the efficacy of these chemicals. The manufacturers publish the results of bacteriological experiments conducted by their own labs, yet that does not bring a substantial credibility for such products. It is in the overall interest of consumers to be aware of the fact that there is tough competition in the business of disinfectants and the efficiency of such products is often vastly exaggerated[3].

Disinfectants are required to have quick and extensive effect, selective toxicity, non-stimulating effect, penetrativeness, apt solubility, resistance to beams and organic substances. In addition, disinfectants should be inexpensive and safe for human but effective on instruments and surroundings. Moreover, disinfectants should neither emanate bad smell nor damage paint. Finally, they should be portable. The widespread

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use of disinfectants and antiseptics has led into emergence and development of cross resistance among microorganisms[4].

Efficiency of disinfectants is determined by temperature, formulation, presence of organic substances, synergy, and concentration. Widespread use of disinfectants may lead to MDR (multi-drug resistance) and development of nosocomial infections and their problematic consequences. It is very important to reduce nosocomial infections by evaluating and introducing appropriate disinfectants. The main objective of this research is to examine bacterial resistance against widespread nosocomial disinfectants[5,6].

MATERIAL AND METHODS

This study is an experimental type and conducted according to the NCCLS 2OO4 standards and instructions and does not need statistical analysis[7]. The following standard species used in this study are obtained from Iran's Industrial and scientific research center.

S. aureus PTCC 1169, *Bacilus cereus* PTCC 1015, *Escherichia coli* PTCC 1037, Enterobacter PTCC 1221, *K. pneumoniae* PTCC 1053, *Bacilus subtilis* PTCC1023, *S. epidermidis* PTCC 1114, *P. aeruginosa* PTCC 1281, *M. smegmatis* PTCC 1307.

The disinfectants studied in this study are; Tetravalent compounds of Amonium (cytrimide), Formalin, Iodine, Deconex, and Microtene. We diluted the disinfectants under study using distilled water and prepared solution with different concentrations; 1/2, 1/3, 1/4, 2%, 5%, 5.5%. Then, the lyophilized bacteria were cultured in Nutrient Broth medium. When the solo cultured microbe had been prepared, the sample was evenly spread over Muller Hinton Agar medium by sterile swap. Then, 6 millimeter discs have been saturated with a 12 micrometer coating of substances and dried in the room temperature and were laid on Muller Hinton medium. Finally, after incubation in 37 degrees Celsius for 24 hours, the bactericidal efficacy of each disinfectant was measured based on the reaction of microorganisms against he test substance and diffusion zone around the test disc.

After a week later the antimicrobial influence of non-growth diffusion zone was measured to confirm the results. The bactericidal effect of the disinfectants could be confirmed if there was no detectable bacterial growth.

To validate the results, some samples using Fyldo platyn rod, were picked up from the non-growth diffusion zone and were cultured in a sterile environment. Consequently, antimicrobial effectiveness of the bactericide would be confirmed if there was no detectable bacterial growth in the disc diffusion zone (Tables 1, 2, 3). At the end, the minimum inhibitory concentration (MIC) of each disinfectant was determined through experiments by measuring the non-growth zone diameter according to the diffusion method[6].

RESULTS

The antimicrobial effect of Iodine on the gram negative bacteria was not detectable. Also, the spore producing bacteria and acid fast were resistant to iodine (Table 1). Some impressions, however, were detected in gram positive bacteria. The Deconex & Citrimid which had been used at certain concentrations as approved by instructions, in the chemical structure of the disinfectants had an average antimicrobial effect. However, a mixture of Microtene 5% and Formalin 37% was very effective and showed bactericidal effect on the gram positive and negative, sporulation & acid fast bacteria.

The antibacterial effect of Deconex at different concentrations on nosocomial bacteria were given in Table 2. If used according to instructions, Deconex can be effective against all bacteria including microbacterium. Table 2 displays minimum inhibitory concentrations (MLC) of Formalin. If used as advised by instructions, Formalin can act against gram positive and negative, sporulation and acid fast bacteria. At the lower concentrations, it can also maintain its bactericidal influence on gram positive, negative and sporulation bacteria.

Minimum inhibitory concentrations (MIC) of Microtene at concentrations mentioned in the table 3, which acts against all bacteria especially acid fast bacteria. And at the lower concentrations, it also shows decent antibacterial efficacy. Table 3 displays minimum inhibitory concentrations (MIC) for a compound of Iodine %10 and Citrimide against test bacteria.

Table 1. Influence of iodine concentrations on test bacteria

Bacteria Disinfectant	Iodine 7.5 %	Iodine 10%	Iodine %10 3/1	Iodine %10 2/1	Iodine %10 4/1
Staphy lococcus aureus	17*	14	15	24	21
Bacillus cereus	26	7	14	0	0
Escherichia coli	17	10	15	0	17
Aerogenes enterobacter	16	15	0	0	0
Klebsiella pneumoniae	14	9	0	15	10
Mycobacterium estigmatism	0	0	0	0	0
Bacillus svbtylys	20	24	13	18	22
Staphylococcus epidermis	18	16	14	18	20
Pseudomonas aeruginosa	29	16	0	0	0

* shows nongrowth zone's diameter (MIC) *

Table 2. Influence of Deconex and formalin concentrations on test bacteria

Bacteria Disinfectant	Deconex	Deconex1/3	Deconex¼	Formalin1/2	Formalin1/3	Formalin1/4
Staphylococcus aureus	19*	10	17	23	37	26
Bacillus cereus	15	7	8	26	21	17
Escherichia coli	12	7	10	15	22	28
Aerogenes enterobacter	9	0	0	23	18	28
Klebsiella pneumoniae	18	5	20	25	32	16
Mycobacterium esmegmatis	0	0	0	15	0	0
Bacillus svbtylys	22	8	15	27	35	19
Staphylococcus epidermis	14	6	7	19	24	21
Pseudomonas aeruginosa	14	12	10	18	14	13

* shows nongrowth zone's diameter (MIC) *

Table 3. Influence of microtone and Iodine concentrations with citrimide on test bacteria

Bacteria Disinfectant	Microtene %2	Microtene %5	Microtene %10	%10 Iodine Citrimide1/2	Iodine %10 Citrimide1/3	Iodine %10 Citrimide1/4
Staphy lococcus aureus	18*	30	17	25	22	21
Bacillus cereus	0	35	25	22	24	20
Escherichia coli	20	21	15	14	18	15
Aerogenes entero bacter	12	30	18	13	9	11
Klebsiella pneumoniae	12	30	15	15	12	0
Mycobacterium esmegmatis	0	25	0	0	0	0
Bacillus svbtylys	0	35	15	19	14	12
Staphylococcus epidermis	12	35	10	17	0	14
Pseudomas aeruginosa	0	21	10	0	17	13

DISCUSSION

Predominant nosocomial infections are highly responsible for patient fatality, economic losses, and prolonged patients' admission in hospitals. Progress in medical science and health care brought some problems, among which the emergence of disinfectant, biocide and antibiotic resistances were the most considerable. Antiseptics and disinfectants are widely used in hospital and other health care centers for disinfecting and cleansing instruments, tools and hospital settings from microbial contaminations[8,9]. Considering diffusion zone size, the disinfectant

compound of Microtene 5% and Formalin 37% proved to be the most effective against all tested bacteria in this research. Hence, this compound is highly recommended as the chosen disinfectant for hospitals and health care centers.

Microtene is not only a concentrated disinfectant but also a strong detergent, free of aldehyde and phenol agents, which is used to disinfect all medical and dental surgery instruments. It also contains a strong anticorrosive agent. This compound is a yellowish liquid which is odorless and tasteless. Moreover, it has a long 5 year expiry date and is free of any aldehyde, acid or phenol substances or any respiratory system stimulants. Also, it is not toxic and does not contaminate the environment. Finally, it is recyclable, safe and can be stored in recyclable polyethylene bottles.

The formaldehyde compounds in the form of liquid formalin and gas, and other aldehydes including glutaraldehyde are used as disinfectants in hospitals on the daily basis. Different concentrations of this substance have been used as advised by instructions showed bactericidal effect against the test bacteria. Once compounded, antimicrobial efficacy of iodine and citrimid increased in this research. *P. aeruginosa*, *K. pneumoniae* and *Staphylococcus epidermis* showed resistance to some concentrations listed in tables. If prepared based on the instructions, the Deconex compound can act effectively against many bacteria including mycobacterium. However, we have to avoid diluting it. This substance in the form of alcoholic spray containing isopropanol and dodecyl amin elements has a strong odor.

Satisfactorily effective in high PH, tetravalent combinations of ammonium (Citrid) are odorless, colorless, not volatile, not toxic and tasteless compounds which do not damage painted surfaces nor does it contaminate the environment. Furthermore, they are resolvable. These compounds are used as disinfectants and antiseptics whose microbicidial effectiveness can be broken-down when exposed to rags, cotton or excessive amount of water.

Increasing usage of disinfectants and antiseptics and encouragements and advertisements by companies to soar consumption on the one hand and the danger of development of resistance by organisms exposed to the disinfectants and the consequences caused by prolonged admission of patients in hospitals. It is in the overall interest of all to ensure that these substances are not used continuously[6,10,11]. Hence, nosocomial infections can be significantly reduced by precise evaluation and introduction of appropriate disinfectants.

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