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Effect of Commercially Available Disinfectants on Wild Type and UV Mutated Nosocomial Pathogen: *Pseudomonas aeruginosa*

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Abstract: The purpose of this study was to evaluate the bactericidal activity of some commercially available disinfectants against hospital bacterial isolates. The organism, *Pseudomonas aeruginosa*, was irradiated for 4 min for the induced mutation and the susceptibility to disinfectants like cetrimide, tincture of iodine, phenol, sodium hypochlorite, alcohol and chlorhexidine gluconate were checked against the wild type culture. The *uvrB* gene mutation in *P. aeruginosa* may lead the opportunistic pathogen to more infectious. The effect of various disinfectants varies with the type of disinfectants, dose of irradiation and density of the culture and concentration of the disinfectants. Sodium hypochlorite, alcohol, phenol had more lethal to wild type. The bactericidal effect was found to be in a range of 4-8 mg mL⁻¹. Cetrimide had great lethality to the both wild type and irradiated one. The effect was found at 2 mg mL⁻¹. Iodine was more effective to wild type (2 mg mL⁻¹) but irradiated one was more resistant even in high concentrations. There was no such effect of chlorhexidine gluconate on both the strain and more growth to be occurred at high concentration. It was further confirmed that gluconate present in the disinfectant was used by the organism as a carbon source.

Key words: Disinfectants, nosocomial pathogen, UV mutation

INTRODUCTION

Nosocomial infections are an important cause of morbidity and mortality all over the world. It has been shown that appropriate environmental hygienic and disinfection practices can be very helpful to hospital infection control. *Pseudomonas aeruginosa* is a Gram-negative, aerobic rod belonging to the bacterial family *Pseudomonadaceae*. It is an opportunistic pathogen, always found in hospital area, causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in patients with severe burns, cystic fibrosis, cancer and AIDS patients who are immunosuppressed. To avoid danger to staff, patients and the environment, prudent use as well as established safety precautions is required. Chemical disinfection of heat-sensitive instruments and targeted disinfection of environmental surfaces are established components of hospital infection control (Markus and Colin, 2005).

The bacterial response to DNA damage caused by UV light, ionizing radiation and chemical agents involves the repair of DNA by several processes. Two products of UV damage, i.e., pyrimidine cyclobutane dimers and pyrimidine 6' to 4' photoproducts, are known to be involved in cellular lethality and both are handled by the nucleotide excision mode of repair in most organisms (Rivera *et al.*, 1996). *Pseudomonas aeruginosa*, which is an important microorganism from both environmental and sanitary points of view, also presents an SOS-like system like *Escherichia coli* (Miller and Kokjohn, 1990). It is significantly more sensitive to UV irradiation than is *E. coli* (McBeth, 1989). In fact, it has been suggested that one or more *recA*-dependent, inducible repair systems present in *E. coli* are lacking or not fully functional in *P. aeruginosa*. The gene responsible for the UV mutation

in *P. aeruginosa* is *uvrB* gene. UvrB protein must be functional in *P. aeruginosa* cells, because a *uvrB*-defective mutant is extremely sensitive to UV radiation (Rivera *et al.*, 1996).

The effect of various disinfectants varies with the type of disinfectants, density of the culture and concentration of the disinfectants (BioSafety Reference Manual, 1995). The lethality and the mutagenicity of the UV are also depended on the types of the organisms, wave length of the UV light, time of exposure etc. Biofilms, forms of surface-adherent, mutation *Pseudomonas* pose an extraordinary challenge to decontamination (Markus and Colin, 2005).

The purpose of this study was to evaluate the bactericidal activity of the commercially available disinfectants like cetrimide, tincture of iodine, phenol, sodium hypochlorite, alcohol and chlorhexidine gluconate against the wild type and the UV mutated *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

The research was carried out at Maulana Azad College, Aurangabad, Maharashtra, India during April-2004-June 2005.

Microorganism

The microorganism was isolated from a burnt patient of Government Medical College, Aurangabad using King's B medium during February 2004 and morphologically and biochemically confirmed as *Pseudomonas aeruginosa* (Bergey's Manual) (Holt *et al.*, 2000).

Lethal UV Dose Assay

The organism was irradiated for different time exposure (1-20 min). The minimum lethal dose was found to be 4 min, where the most the characteristics like pigment production, generation time etc were affected.

Disinfectants Susceptibility Assay

In a series of test tubes 1 mL of corresponding disinfectants having concentration 2, 4, 8, 12 and 16 mg mL⁻¹ were taken along with 3 mL of culture (both wild type and irradiated) and 1 mL of minimal media and incubated at 37°C for 24 h. After 24 h the optical density at 600 nm was taken against a blank (1 mL water + 1 mL minimal media + 3 mL culture).

RESULTS

The bactericidal activities of different disinfectants on irradiated and wild type culture of *Pseudomonas aeruginosa* was presented in Table 1. The results shown the differences of optical density after the 24 h growth (Final OD- Initial OD).

Table 1: Optical density at 600 nm at different concentrations of disinfectants after incubating at 37°C for 24 h

		Optical density at 600 nm on different concentration				
Disinfectants	Culture	2 mg mL ⁻¹	4 mg mL ⁻¹	8 mg mL ⁻¹	12 mg mL ⁻¹	16 mg mL ⁻¹
Cetrimide	Pseud _{irrad}	-0.01	-0.05	-0.06	-0.07	-0.07
	Pseud _{WT}	-0.01	-0.02	-0.05	-0.07	-0.03
Tincture of Iodine	Pseud _{irrad}	0.35	0.23	0.22	0.13	0.13
	Pseud _{WT}	-0.02	-0.03	-0.04	-0.04	-0.07
Phenol	Pseud _{irrad}	0.07	0.06	0.09	0.08	0.07
	Pseud _{WT}	0.06	0.02	0.03	0.01	-0.02
Sodium Hypochlorite	Pseud _{irrad}	0.07	0.01	-0.06	-0.05	-0.07
	Pseud _{WT}	0.11	0.03	-0.07	-0.08	-0.12
Alcohol	Pseud _{irrad}	0.07	0.03	0.02	0.02	0.02
	Pseud _{WT}	0.05	0.02	0.02	-0.01	-0.02
Chlorhexidine Gluconate	Pseud _{irrad}	0.14	0.22	0.38	0.37	0.42
	Pseud _{WT}	0.11	0.12	0.18	0.25	0.41

N.B.-Pseud_{irrad} = *Pseudomonas aeruginosa* Irradiated, Pseud_{WT} = *Pseudomonas aeruginosa* wild type

Sodium hypochlorite, alcohol, phenol had more effective to wild type. The maximum effect was found in a range of 4-8 mg mL⁻¹. Cetrinide had great bactericidal to the both wild type and irradiated one. Iodine was more effective to wild type (2 mg mL⁻¹) but irradiated one was more susceptible to the disinfectants even in high concentration. There was no such effect of chlorhexidine gluconate on both the strain and more growth was occurred at high concentration. It is further confirmed that gluconate can also used as carbon sources for the growth of *Pseudomonas aeruginosa*.

DISCUSSION

Radiations are used extensively for disinfecting enclosed areas such as entry ways, operation theaters, laboratories but it may also causes mutation that may lead to the nosocomial pathogen to more dangerous. They even tolerate the effect of disinfectants commonly used in the hospitals. Ultraviolet radiation (UVR) of wavelengths in the region of 260 nm is strongly absorbed by the purine and pyrimidine bases in nucleic acids. The main effect is believed to involve the formation of pyrimidine dimers (particularly thymine dimers) in DNA; covalent bonds are formed between the 5, 6-positions of two adjacent pyrimidines-a cyclobutane ring being formed between the two residues. The lethal effect of phenol, cetrinide, a quaternary ammonium compound (quat) and chlorhexidine is due to their capacity to cause cell membrane damage, thus releasing cell contents and causing lysis. Low concentrations of phenol can also precipitate protein and membrane-bound oxidases and dehydrogenase are irreversibly inactivated by concentrations of phenol that are rapidly bactericidal for the organism. Iodine combines with cellular proteins (e.g., tyrosine is irreversibly iodinated). Alcohol act by denaturing bacterial proteins. The antimicrobial action of hypochlorite is believed to depend mainly on the liberation of nascent oxygen ($\text{HOCL} \rightarrow \text{HCl} + \text{O}$) which oxidizes cell constituents.

In this study it was found out that the UV mutated strain of *Pseudomonas aeruginosa* was more resistant to commercially available disinfectants with respect to wild type. It gave a clear idea that the mutated opportunistic nosocomial pathogen might be more harmful to the immunosuppressed patients in hospital. New disinfectants, mainly peroxygen compounds, show good sporicidal properties and will probably replace more problematical substances such as chlorine-releasing agents. As emerging resistant pathogens will challenge healthcare facilities in the future even more than at present, there is a need for well-designed studies addressing the role of disinfection in hospital infection control (Markus and Colin, 2005).

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