Bactericidal Effect of Direct and Filtered Ultraviolet C Through Transparent Plastic Sheet on Gram Negative Bacilli – An In-Vitro Study

Bhamini Krishna Rao*,1, Pramod Kumar1, Sugandhi Rao1, Jagdishchandra K1, Pratijna Suhasini2, Asha Kamath3

*1Department of Physiotherapy, Manipal College of Allied Health Sciences, Manipal University, Manipal – 576104, Karnataka, India
2Dr. M.V. Shetty College of Medical Lab Technology, Mangalore. India
3Department of Community Medicine, KMC Manipal, India
E-mail of Corresponding Author: bhaminikr@gmail.com

Abstract

Wound infection (with both Gram-positive and Gram-negative organisms) is a major problem in delayed wound healing. Persistence of these organisms in the wound delays considerably the healing time and increases length of hospital stay. Ultraviolet-C has shown to effectively eliminate these organisms (bactericidal effect) in few laboratory, animal and human studies and has shown promising role in promoting wound healing. Several wound dressing methods have been used to accelerate the rate of wound healing. Among them, Limited Access Dressing (LAD) utilizes intermittent negative pressure dressing using a transparent plastic cover combined with moist wound healing principle, minimizes need for regular change of dressing. Thus, the present study was conducted to explore the in-vitro bactericidal effect of direct and filtered UVC through transparent plastic sheet on Gram-negative bacilli.

Keywords: Ultraviolet rays, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli

1. Introduction

Ultraviolet rays (200-290nm UVC) have been shown to have bactericidal effect1,2,3, enhanced growth of epithelial cells and promotes granulation tissue formation which promotes wound healing4,5. Bactericidal effect of ultraviolet rays was studied in different in-vitro study methods1,2 and on common wound infecting organisms like Staphylococcus, Escherichia coli, Pseudomonas aeruginosa and fungal strains by different researchers2,3. UVC produces intra–strand cyclobutane–type pyrimidine dimers in Deoxyribonucleic acid (DNA). These photoproducts can be lethal (not viable and looses the ability to replicate) and ultimate fate of irradiated cells depends upon the ability of the cells to repair the UV induced damage6,7. Many researchers have identified the prevalence of Staphylococcus aureus, Klebsiella species, Pseudomonas aeruginosa and Escherichia coli8-11 in different types of wound infection. Unlike in the West, in India multi drug resistant gram-negative bacteria were found to have always been dominant in the wounds infections12. The widespread use of antimicrobials in the management of many infections including wound infection has led to major concern of MDR microorganisms13. Certain well established principles are followed in wound care like debridement and cleansing, maintenance of a moist environment, providing topical agents to reduce bacterial load and promote healing, prevention of further injury along with systemic antibiotics. Long term use of topical antimicrobials like iodine, hydrogen peroxide, silver preparations even though reduces the bacterial burden but have many side effects14,15. The widespread use of negative pressure wound therapy (NPWT) is a relatively a new trend in wound care16. However, in extensive open wounds it may be difficult to use NPWT. In these instances, a modification using sterilized plastic covers and two drainage tubes – a technique called Limited Access dressing (LAD)17,18 has been used successfully in more than 1000 patients in 2009 at Kasturba Hospital (Manipal, INDIA). LAD uses intermittent negative pressure (~30 mmHg for 30 minutes) through the tube(s) and maintains moisture at the wound site (for 3 hours 30 minutes without negative pressure) with the use of transparent polythene material (a total of 21 hours of moist dressing and 3 hours of negative pressure dressing in a 24- hour period, case report19, case series20).

Previous studies have shown that UVC is possessing bactericidal effect against multi drug resistant bacteria in in-vitro 21 and in
human studies\textsuperscript{21,22}. In recent years, increase in the multi drug resistant bacteria\textsuperscript{13–15} and understanding the benefits of both LAD (cost effectiveness, transparency, reduced chances of anaerobic infection and reduced smell) and UVC on bacterial growth have increased. In an empirical study\textsuperscript{23}, it was reported inconclusive results regarding the bactericidal effects of UV light on a wound covered with the transparent dressing. Though UVC has proven as definitive adjunct in wound healing, of late, not many studies have been directed in this regards. Using this information, authors designed this prospective in -vitro study to ascertain the bactericidal effect of UVC, direct and filtered through a transparent plastic sheet, on gram-negative bacilli.

2. Materials and methods:

2.1: An In-vitro study was conducted on following Gram-negative bacilli: Following organisms (clinical isolate) were collected from the pus section of Department of Microbiology KMC Manipal. Organisms were identified according to standard microbiological methods\textsuperscript{24} and tested for antibiotic sensitivity by disk diffusion method and sensitivity pattern recorded. 

\textit{Pseudomonas aeruginosa}, \textit{Klebsiella pneumoniae}, \textit{Escherichia coli} (Sensitive, moderately sensitive and resistant pan drug resistant pattern, designated as 1, 2, 3, 4) were used.

2.2: Preparation of bacterial cultures: A standard inoculum for the study was prepared from the isolated bacterial colony as follows: A single bacterial colony was inoculated into peptone water and incubated for six hours at 37°C. After six hours, turbidity of the peptone water was standardized to McFarland’s standard tube to get final concentration of 10\textsuperscript{5} organisms /ml. One standard loop (4mm size, holding 0.01ml of inoculum) of the broth culture was inoculated to MacConkey agar plate by semi quantitative method by continuous streaking without intermittent heating on four quadrants\textsuperscript{25}.\textsuperscript{26}

2.3: UVC dosimetry: Ultraviolet violet rays (UVR) source Endolamp 47428 (Enraf Nonius Holland Figure 1) which emits 74% of UVC 254nm (Spectrum analysis, Figure 2), UVB: 5%, UVA 2.5% and visible light 18.5% (percentage of emission was obtained from the company manual). Unit was regularly monitored for the output (5mJ/s/cm\textsuperscript{2} at 10 cm of distance) by using Broadband power energy meter (13PEM 001 Melles Griot).

2.4: UVC exposure protocol: During the study each culture medium was exposed to direct and filtered UVC through plastic sheet ( thickness 0.15mm) kept as interface between plate and UV source being regularly used for limited access dressing for 5, 10, 15, 20, 25, 30 seconds. The distance between the source and plate for the in vitro study was maintained at 10 cms (as per the instruction manual of machine) by use of indigenously designed (by investigator using thermocol piece) rigid box and the applicator was kept on the box before irradiation. One culture plate with bacterial colony streaked was kept as sham control (was not exposed to UVC) while 18 plates with bacterial growth were exposed to UVC (3 plates containing same bacteria for each duration). Experiment was repeated thrice with different organisms with each antibiotic susceptibility patterns. (Sensitive, moderately sensitive, resistant and pan drug resistant, designated as 1, 2, 3, 4).

2.5: Incubation and bacterial growth: All plates (control and UVC exposed) were incubated at 37°C for 24 hours and observed for growth of bacteria. The duration of the exposure required to cause no growth of the organism was noted. Media growth patterns were assessed. No growth was considered as zero. Scanty growth (first quadrant only), colonies were counted and then multiplied by 10029,30. Scanty growth (first quadrant only) and <10 colonies or <1,000 CFU/mL. Scanty growth and approximately 50 colonies or 5,000 CFU/mL. Scanty growth and 50–100 colonies or 10,000 CFU/mL; 25,000 CFU/mL growth or difficult to count in first quadrant. Growth in first and second quadrant and 50,000 CFU/mL; first, second and third quadrant growth and 75,000 CFU/mL; and >105 CFU/mL and growth in all 4 quadrants.

2.6: Data collection and analysis: Specimen collection and preparation of bacterial culture was conducted at Department of Microbiology (JK, PGR) and BKR conducted UVC exposure to the inoculated agar plate and, PS provided interpretations of the results.

3. Results

Using direct UVC, the exposure time required to achieve no growth (complete eradication) ranged from 15 seconds for \textit{Escherichia coli} to 25 seconds for \textit{Klebsiella pneumoniae}. Filtered UVC even until 30 seconds had no effect on bacterial growth(full growth/105 CFU/mL). This shows that direct UVC has good bactericidal effect on the organisms studied and plastic sheet of 0.15 mm effectively blocks UVC (Table 1).
Our study on *Pseudomonas aeruginosa*, 100% eradication was achieved (20 seconds) with source at 10 cm distance and 5mW/cm² UVC energy. Previous study3 reported 100% eradication of *Pseudomonas aeruginosa* by 30 seconds (average output, 15.54mW/ cm² at 1-inch distance). Different antibiotic susceptibility patterns of *Pseudomonas aeruginosa* did not have any influence on UVC sensitivity. The time required to obtain 100 % bactericidal effect on *Klebsiella pneumoniae* was 25 seconds. Our study showed that longer duration of exposure was required for 100% eradication of pan drug resistant *Klebsiella pneumoniae* than their sensitive strain. This indicated increased UVC resistance of the antibiotic resistant *Klebsiella pneumoniae*. Similar finding was reported in a study36 which showed that UV radiation (exposure duration of 20 seconds) reducing the number of viable cells was 30%-62% for resistant *K. pneumoniae* and 43%-66% reduction for the parent drug-susceptible strains. There was no mention about the wavelength and intensity of UVR in their study.

We have obtained bactericidal effect for *Escherichia coli*, which varied, from 5-15 seconds for different antibiotic sensitivity patterns. Our results indicate that the antibiotic resistant organisms were also resistant to UVC exposure. This is similar to study36, which reported with 20 seconds of irradiation with UV light, a 14-69% reduction in the number of viable cells for resistant E. coli strains, compared with 56-79% reduction for susceptible strains. Another in-vitro study1 showed the effect of Kromayer (UVR) lamp model 10, (254-436nm) on *Pseudomonas aeruginosa* and *Escherichia coli* with dosage varying from E2, E3, E4 at 10 cm distance. Authors concluded these bacterial strains were not viable beyond E4 dose. However, in this study authors have not described duration of exposure and percentage emission of different wavelengths of UVR.

In previous in-vitro study conducted by our team on gram-positive cocci showed, higher UVC sensitivity compared to the results of present study on gram-negative bacilli (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). This observation is not consistent with results of a previous study38. The probable reason for the different UVC sensitivity between Gram-positive and Gram-negative bacteria could be ascribed to the morphological differences between these microorganisms. Gram-negative cells have a complex additional outer barrier that...
In vitro filtered effect of UVC through plastic sheet on common wound infecting organisms: In our study, there was no bactericidal effect of filtered UVC. Then we conducted an experiment to see the amount of radiation passing through the plastic sheet used in this study by UV photometer (UV 1700 series) at Department of Biotechnology, Manipal University. This showed highest optical density of 0.079 at 292 nm. Optical densities showed a reduction from 296 nm till 400 nm. This indicates that complete absorption of the rays were seen below 296 nm. This may be the reason for filtered UVC not having any bactericidal effect. In 1984 a study reported UVR can be applied through the transparent dressing. Then in 2001 a research group reported UVR did not show any filtered effect, our results were consistent with reports of this group.

5. Conclusion
Direct UVC (Endolamp 474) has bactericidal effect in short duration (15 seconds) of exposure for Gram-negative bacilli but filtered UVC is ineffective. This beneficial effect of direct UVC in short duration minimizes the bacterial load and simultaneously reduces side effect related to exposure duration. Escherichia coli is more sensitive to the effects of UVC than Pseudomonas aeruginosa and Klebsiella pneumoniae. More resistant patterns of Klebsiella pneumoniae and Escherichia coli was also UVC resistant, but similar effect was not observed with Pseudomonas aeruginosa. Also plastic sheet of 0.15 mm thickness may be used to protect the surrounding skin to protect from exposure during therapy.

References
1. High AS, High JP. Treatment of infected skin wounds using ultraviolet radiation: An in-vitro study. J Chartered Soc Physiother 1983; 69(10): 359-60.
2. Sullivan PK, Conner-Kerr T and Smith S. The effects of UVC irradiation on group A streptococcus in vitro. Ostomy Wound Manage 1999; 45(10): 50-8.
3. Sullivan PK, Conner-Kerr T. A comparative study of the effects of UVC irradiation on select procaryotic and eucaryotic wound pathogens. Ostomy/Wound Manage 2000; 46(10): 44-50.
4. Nussbaum EL, Biemann I, Mustard B. Comparison of ultrasound/ultraviolet: Cand laser for treatment of pressure sores in patients with spinal cord injury. Physiother 1994; 74(9): 812-5.
5. Wadsworth H, Channugam APP. Eelectro-physical agents in physiotherapy therapeutic and diagnostic use. 2nd ed. Science Press; p 148-81.
6. Hamkalo BA and Swenson PA. Effects of ultraviolet radiation on respiration and growth in radiation resistant and radiation sensitive strains of Escherichia coli B. J Bacteriol 1969 Sept; 815-23.
7. Michlovitz SL. Thermal agents in rehabilitation, 3rd ed. Philadelphia: FA Davis Company; p 269-75.
8. Murugan S, Mani KR, Uma Devi P. Prevalence of Methicillin Resistant Staphylococcus aureus among diabetes patients with foot ulcers and their antimicrobial susceptibility pattern. J Clin Diag Res [serial online] 2008 August [cited: 2010 Mar 27]; 2: 979-84. Available from http://www.jcdr.net/back_issues.asp?issn=0973-709x&year=2008&month=Augustvolume=2&issue=4&page=979-984&id=306
9. Gupta Naveen, Gautam Vikas, Saini Santosh, Singh Lokveer, Arora Deshraj. Prevalence of multi drug resistant bacteria in wound infections. J Infect Dis Antimicrob Agents 2002; 19: 111-7.
10. Anbumani N, Kalyan J, Mallika M. Epidemiology and microbiology of wound infections. Indian J Pract Doct 2006; 3(5): (page no).
11. Ekta Bansa, Ashish Garg, Sanjeev Bhatia, Attri AK, Jagdish Chander. Spectrum of microbial flora in diabetic foot ulcer. Indian J Pathol Microbiol 2008; 51(2): 204-8.
12. Changing microbiological profile of pathogenic bacteria in diabetic foot infections: time for a rethink on which empirical therapy to choose? Diabetologia 2011; 54: 58–64. DOI 10.1007/s00125-010-1893-7
13. Dessmon YH Tai, Lay Hong Goh. Challenges in Managing Gram-Positive Bacteria Resistance. Medical Progress 2002 Dec: 15-21.
14. Duc Q, Breetveld M, Middelkoop E, Scheper RJ, Ulrich MM, Gibbs S. A cytotoxic analysis of antiseptic medication on skin substitutes and auto graft. Br J Dermatol 2007; 157(1): 33-40.
15. Poon VK, Burd A. In vitro cytotoxicity of silver: implication for clinical wound care. *Burns* 2004; 30(2): 140-7.
16. Carol Harvey. Wound healing *Orthopaedic Nursing;* 2005 Mar/Apr; 24(2); 143. ProQuest Medical Library.
17. Kumar P. Limited access dressing. *Wounds* 2008; 20(2): 49-59.
18. Kumar P. Limited Access Dressing for aggressive wound management. 38th National Annual Conference of Association Plastic Surgeons of India (APSICON 2003). Sep 10-13. Ooty, Tamilnadu, India, 2003.
19. Kumar P. Limited Access Dressing and Maggots. *Wounds* 2009; 21(6):150-2.
20. Kumar P, Ankur S. The Limited Access Dressing for Damage Control in Trauma Patients. *Wounds* 2010; 22(7): 188–92.
21. Conner-Kerr TA, Sullivan PK, Gaillard J, Franklin ME, Jones RM. The effects of ultraviolet radiation on antibiotic-resistant bacteria in vitro. *Ostomy Wound Managt* 1998; 44(10): 50-6.
22. Thai TP, Houghton PE, Keast DH, Karen E, Campbell RN and Woodbury MG. Ultraviolet Light C in the Treatment of Chronic Wounds with MRSA: A Case Study. *Ostomy Wound Managt* 2002 Nov; 48(11): 52-60.
23. Thai TP, Keast DH, Campbell KE, Woodbury MG, Houghton PE. Effect of ultraviolet light C on bacterial colonization in chronic wounds. *Ostomy/Wound Managt* 2005; 51(10): 32-45.
24. MacKinnon JL, Cleek PL. Therapeutic penetration of ultraviolet rays through transparent dressing. *Phys Ther* 1984; 64: 204.
25. Collee JG, Marr W. Specimen collection, culture container and media. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie & McCartney Practical Medical Microbiology, 14th ed. New York: Churchill Livingstone; 1996. P 95-149.
26. Maki DG, Weise CE, Sarafin HW. A semiquantitative culture method for identifying intravenous catheter related infections. *New Engl J Med* 1977; 296(23): 1305-9.
27. Sitges-serra A, Linares J. Limitations of semiquantitative method for catheter culture (letter). *J Clin Microbiol* 1988; 26: 1074-6.
28. Forbes BA, Sahm DF, Weissfeld AS. Specimen management. In. Bailey & Scott’s Diagnostic Microbiology, 12th ed. Mosby Elsevier: Missouri; 2007. p 62-77.
29. Endolamp 474 Operators Manual, 1986.
30. Padmini JW, Parasuraman M Urine Chapter X. In: Myer’s and Koshi’s manual of diagnostic procedures in medical microbiology and immunology/serology. Compiled by faculty department of clinical microbiology Christian Medical College and Hospital, Vellore Tamilnadu, India; 2001: p 57-62.
31. Exposure to artificial UV radiation and skin cancer/views and expert opinions of an IARC Working Group that met in Lyon, France 27-29 June 2005, published in 2006.
32. Occupational standard for exposure to ultraviolet radiation, National Health and Medical Research Council 1989, Radiation Health Series No.29. Canberra: NHMRC.
33. Occupational Exposure to Ultraviolet Radiation. Radiation Protection Series Australian Radiation Protection and Nuclear Safety agency. Publication No. 12 December 2006.
34. Conner-Kerr TA, Sullivan PK, Gaillard J, Franklin ME, Jones RM. The effects of ultraviolet radiation on antibiotic-resistant bacteria in vitro. *Ostomy Wound Managt* 1998; 44(10): 50-6.
35. Johnson RG. US Patent 6, 283, 986, 2001 - Google Patents.
36. Marchese A, Gualco L, Debbia EA, Schito GC, Schito AM. In vitro activity of fosfomycin against Gram-negative urinary pathogens and the biological cost of fosfomycin resistance. *Int J Antimicrob Agents* 2003; 22: S53- 9.
37. Bhamini K Rao, Pramod Kumar, Sugandhi Rao, Bimala Gurung. Bactericidal Effect of Ultraviolet C (UVC), Direct and Filtered Through Transparent Plastic, on Gram-positive Cocci: An In Vitro Study. *Ostomy Wound Managt* 2011; 57(7): 46–52.
38. Blatchley ER, Peel MM. Disinfection by Ultraviolet radiation. In: Seymour S Block, editor. Disinfection, Sterilization and Preservation. 5th ed. Lippincott Williams and Wilkins: 2001. p 823-51.
Table-1 shows effective time to cause no growth for Gram-negative bacilli

| Pathogen            | Time in seconds (Direct) | Time in seconds (filtered) |
|---------------------|--------------------------|----------------------------|
| *Pseudomonas aeruginosa* | 20 (no growth)            | 105 CFU/ml till 30 seconds of exposure |
| *Klebsiella pneumoniae* | 25 (no growth)            | 105 CFU/ml till 30 seconds of exposure |
| *Escherichia Coli*   | 15 (no growth)            | 105 CFU/ml till 30 seconds of exposure |

Table-2: Effect of direct UVR (254nm) on *Pseudomonas aeruginosa*

*No growth in the agar plate is shown as (-)

Table-3: Effect of direct UVR (254nm) on *Klebsiella pneumoniae*.

*No growth in the agar plate is shown as (-)

Table4: *In vitro* direct effect of Endolamp 474 ultraviolet rays on *Escherichia coli* with different antibiotic susceptibility pattern

*No growth in the agar plate is shown as (-)