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The Effect of Ultra Violet Radiation on Methicillin Resistant *Staphylococcus aureus* and Multi-drug Resistant *Acinetobacter baumannii*

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Abstract:*The problem caused by multidrug-resistant bacteria is on the increase worldwide creating the need to look into all measures aimed at control. Isolates of MRSA and Acinetobacter baumanniiwereobtained from the microbiology laboratory of the King Faisal University College of Medicine. They were sub-cultured on blood and MacConkey agar respectively and exposed to ultra violet radiation for 5, 10, 15, 20, 25, 30 seconds and the experiment was carried out in triplicates. Two sets of control experiments were set up, control one had the bacteria inoculated petri dish covered before exposure to UV radiation and the second control was not exposed to UV radiation. All experimental UV radiation exposed bacterial cultures and the controls were then incubated at 37°C for 24 hours. The number of growth colonies on the control plates was used to compare the zones of inhibition of the experimental treatment. The obtained results showed growth inhibition of MRSA and Acinetobacter baumannii that increased with the increase in the length of time of UV radiation exposure, thus the maximum growth inhibition was at 30 seconds of exposure while the minimum was the specimen subjected to 10 seconds of exposure, with no significance inhibition within 5 seconds length of exposure.This study demonstrates the toxic effect of ultraviolet radiation in the reducing the growth rate of multi drug resistance bacteria. However, the surviving multi bacterial colonies did not lose their multi-drug resistance characteristics.*
Key words:*Ultra violet radiation, MRSA, Multidrug resistant, Acinetobacter baumannii*

I. Introduction

The effect of ultra violet radiation on bacteria has received attention in recent times by many researchers [1, 2, 3]. The reasons for such investigations have differed between researchers, some of which have looked into the effect of different sources and wave lengths of Ultra Violet radiation on microbial control. However, Michelle et al., [4] attributed this increasing interest to be as a result of the intractable problem caused by microbial antibiotic resistance. The problem caused by multiple drug resistance bacterial strains is on the increase and has remained a major public health issue. The time taken for the development of new antibiotics to tackle these new bacteria strains is not as fast as the rate at which they are needed. It is therefore pertinent to look into all measures aimed at control probably through the use of materials readily available in nature and one of such material is UV radiation. Ritter, et al.,[5]were of the view that the problem of multi-drug resistant bacteria pathogens persists in the 21st century. When appropriate, if safety precautions are taken, ultraviolet lighting appears to be an effective way to lower the risk of infection in the operating room during major surgeries. Schrier, et al.,[6] investigated antimicrobial efficacy of riboflavin and ultraviolet light on *Staphylococcus aureus*, MRSA, and *Pseudomonas aeruginosa* and reported that riboflavin in combination with UV light is an effective modality to eradicate MRSA, and *P. aeruginosa*. They were of the view that UV light as a monotherapy was not effective in bacterial inhibition. A review by Dai, et al.,[7]mentioned that with appropriate doses, UVC may selectively inactivate microorganisms while preserving viability of mammalian cells and promote wound healing. UVC is also found in animal studies to be less damaging to tissue than UVB. Even though UVC may produce DNA damage in mammalian cells, it can be rapidly repaired by DNA repair enzymes. A study [8] suggested that UVC light reduced the bacterial burden of *P. aeruginosa* and *S. aureus* in the infected mouse cutaneous wounds. Another study [9] on*Acinetobacterbaumannii* infections reported comparable outcomes. Also, Burnside, et al., [10] reported that using the concept of attenuating pathogenic effect of the bacteria, a new modality of vaccination has been tried recently in mice models with a UV-irradiated genetically attenuated mutant of *S. aureus* vaccine that was found to provide protection against subsequent systemic infection with virulent methicillin-sensitive or methicillin-resistant *S aureus*. Nerandzic, et al., [11] tested a novel environmental disinfection technology with ultraviolet radiation that rapidly kills Clostridium difficile spores and other healthcare-associated pathogens on surfaces, and concluded that the results were promising. The present study investigates the effect of ultraviolet radiation on Multi Drug Resistance Bacteria strains of Methicillin-resistant Staphylococcus aureus (MRSA) and *Acinetobacter baumannii* with the view of further highlighting the possibility of application of the findings in the control of Multi-drug resistant bacteria in control and treatment measures.

II. Materials And Methods

Bacterial isolates:

Methicillin resistance *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii*specimenswere obtained from microbiology laboratory of college of medicine, KFU. They had been isolated through basic microbiological techniques and confirmed through biochemical techniques. MRSA were subcultures in blood agar, and *Acinetobacter baumannii* were subcultures in MacConkey agar. A total of eight plates of each pathogen were prepared. For each of the isolates, two sets of control experiments were set up. Control 1 was not exposed to UV radiation, while for control2, the bacteria inoculated Petri dish was exposed to UV radiations with the lid on the dish. The other six plates were labeled according to their time of exposure 5, 10, 15, 20, 25, 30 seconds. The eight plates were exposed for a certain time; 5, 10, 15, 20, 25, 30 seconds and incubated aerobically at 37^0C for 24 hours. The experiment was repeated in triplicates.

Determination of Bacterial survival time

The bacteria survival was monitored by colony count of both the controls and the experimental. The number of colonies on the control plates was used to compare those of the experimental treatment. The method for calculating percentage of surviving bacteria is as described by Djurdjevic-Milosevic et al., [2]using the formula; $S(\%) = (N – N_0) /N_0 X 100$.

Antibiotic Sensitivity Test.

Isolates that survived the exposures to UV radiation were subjected to Antibiogram test by plating them on nutrient agar and exposing them to the antibiotics of which they had been previously tested and showed resistance. Sensitivity test was carried out in the 25 up to 30 second exposed isolates for both pathogens in order to examine resistant of the isolates after UV exposure. MRSA isolates were tested for sensitivity for Methicillin and Vancomycin, while *Acinetobacter baumannii* was tested against the Carbapenems.

PCR analysis

Polymerase chain reaction (PCR) amplification was used to detect the presence of the OXA-40 Carbapenemase genes.

III. Statistical Analysis.

Readings were calculated as percentage survival. And the results were analyzed using Students T-Test at confidence interval 95% and $P <0.05$.

IV. Results

The results on the effect of Ultra violet radiation on Methicillin resistant *Staphylococcus aureus* and *Acinetobacter baumannii* is shown in figure 1. The figure shows that there was increased inhibition with the increase in the length oftime ofUV radiation exposure for both organisms, thus the maximum growth inhibition was at 30 seconds of exposure while the minimum was the specimen subjected to 10 seconds of exposure.Thecomparison of the percentage inhibition of bacterial growth by the UV radiation on the multidrug resistant bacteria is shown in fig. 3. The inhibition of MRSA is found to be statistically significant ($P= 0.12$), CI 95% (19.11, 96.39), as well as for *A. baumannii* ($P=0.25$), CI 95% (10.04, 98.96). The effect of exposure of MRSA and *A. baumannii* to ultra violet radiation at 5 and 10 seconds is comparable and shows no significant difference between the two strains (P value > 0.05), At the time of 15, 20 25 secs, the effect was higher with the *A. baumannii* group, P value≤ 0.05, while at the time of 30 seconds post UV exposure, the effect was higher for MRSA (P value = 0.007), CI 95% (78.29 103.71).

The results obtained also showed that UV radiation did not alter the antibiotic susceptibility of the test organism. *Staphylococcus aureus* was still resistant to Methicillin and *A. baumannii* remained resistant to the Carbapenems. The results presented in figure 3 shows the ultraviolet radiation did not affect the genes contributing to resistance in *A. baumannii* isolates. For all the isolates, the OXA-40 Carbapenemase genes were not affected irrespective of the length time of exposure to ultraviolet radiation.

Fig. 1: Showing Percentage Inhibition of MRSA and *Acinetobacter baumannii* post Ultra violet radiation treatment

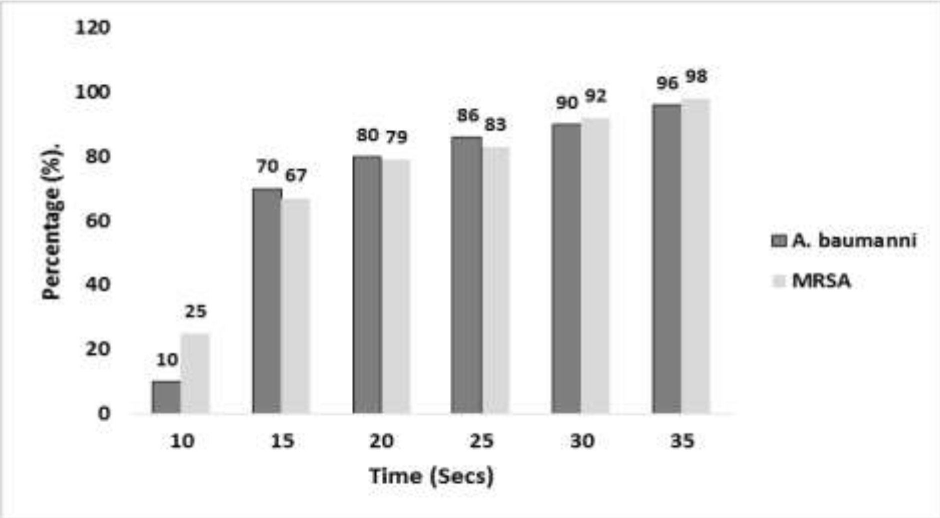


Fig. 2: A Comparison of Mean Growth Inhibition of Isolates by UV radiation

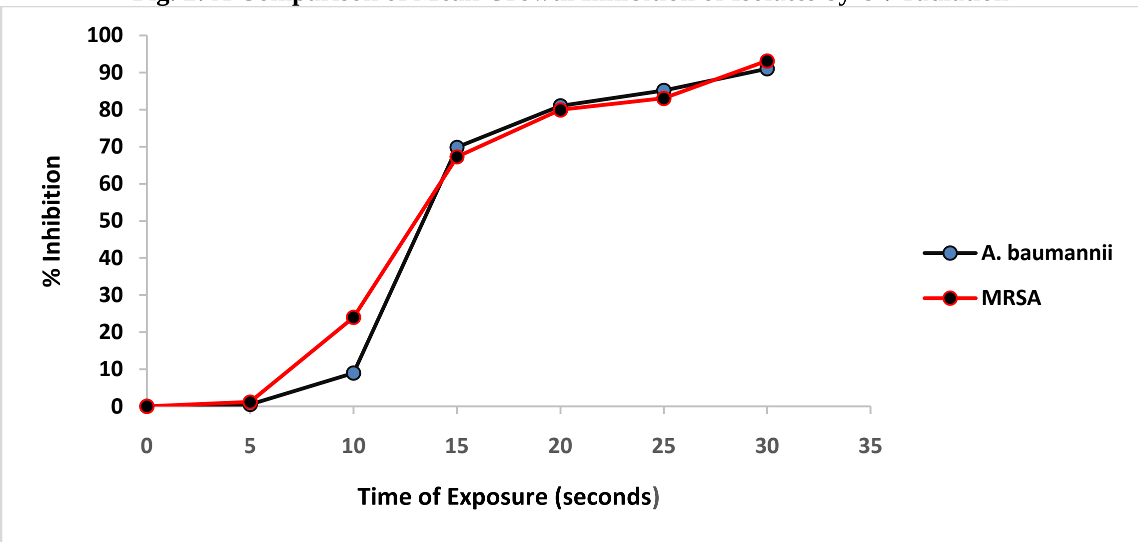
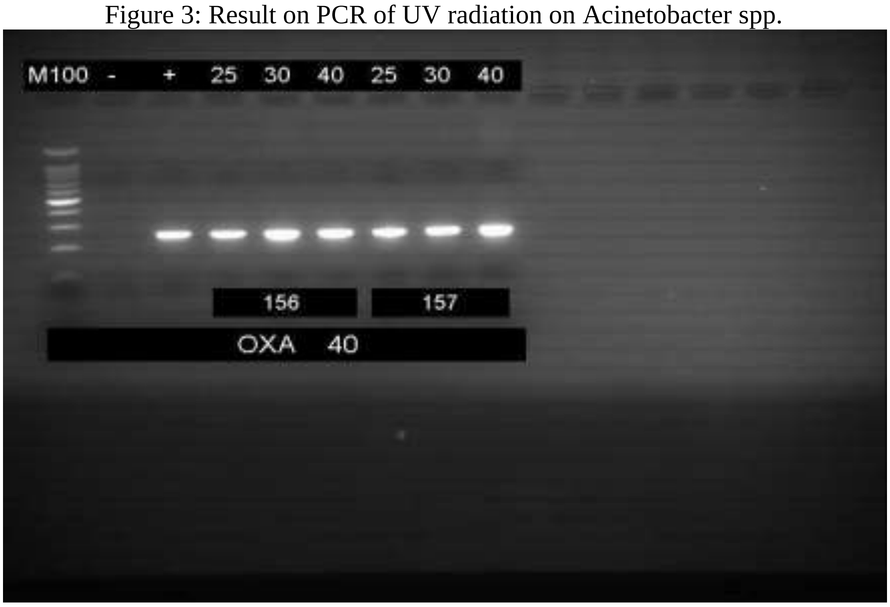


Figure 3: Result on PCR of UV radiation on Acinetobacter spp.



V. Discussion

In the present study, ultra violet radiation is seen to reduce microbial growth of multidrug resistant bacteria. Inhibition of bacterial growth by UV radiation has been reported by workers such as Angelica et al., [1], DM Djurdjevic-Milosevic et al.,[2], Paul et al., [3]and Sullivan et al., [12, 13].Results in figure 1, shows that the two bacteria isolates under consideration, varied in the rate at which they were inhibitedby UV treatment. That twenty five percent of MRSA growth were inhibited 10 seconds post exposure while for *A. baumannii*, only ten percent growth inhibition at this time probably indicates a difference inresponse by the different bacteria isolates. Earlier studies [14] showed a 99.9% MRSA growth inhibition at 60 seconds post exposure to UV radiation and complete inhibition at 120 seconds post exposure contrary to the present findings were 92% of the isolates were eradicated at 30 secs post UV exposure for MRSA. It is therefore unlikely that the MRSA isolate in the present investigation would have survived for up to 120 seconds post UV exposure. This difference could be due to difference in experimental procedures or the sources of UV radiation as well as probable difference in regional bacterial strains. Also despite the fact that only 90% growth inhibition was obtained for *A. baumannii* could indicate a species related response to ultra violet radiation. However, Michelle et al., [4] reported that inactivation of gram-negative bacteria was much less than that for gram-positive bacterial. This Maclean et al.[15]explained from an earlier investigation could be due to differences in the sources as well as the wavelength of the UV radiation. Earlier, Beck [16] showed a growth inhibition of between 40 – 75% for *Serratia marcescens*at 15 secs post UV exposure, a 75 – 90% inhibition at 30 secs post exposure and 95 – 99 percent clearance one minute post exposure thus indicating a species related differences in response to ultra violet radiation treatment. One thing however appears common to all the isolates and that is the reduction of growth with an increase in the time of radiation which is not unexpected. This ultraviolet light exposure seemed not to have affected the genetic composition of the bacteria isolates as their antibiotic susceptibility pattern remained unaltered post exposure to radiation. Thispoints to the fact that growth might have been inhibited with no visible change in the genetic content of the bacteria as show from the PCR analysis results in figure 2. Similar findings had been reported [17]. They stipulated that exposure to UV does not upregulate antibiotic resistance and that radiation does not lead to a more antibiotic-resistant population either. There appears however, to be a need for further investigations into the appropriate tolerable doses of ultra-violet radiation that would ensure the inactivation of all resistant bacteria strains in infected people and at the same time remain safe and harmless to mammalian cells. It can therefore mean that skin tanning, either by exposure to sunlight or through the use of UV sun beds could be an advantage in the control of multidrug resistant bacteria as their growth is inhibited.

VI. Conclusion.

The present investigation therefore shows that ultra violet radiation inhibits the growth of MRSA and multidrug resistant *A. baumannii* thus suggesting the need to look into such as added techniques in treatment and control measures. There is however a need for further investigations.

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Conflict of interest:

The authors declare that there are no conflict of interest

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