

# An *in vitro* Study on the Apoptosis Inducing Effects of Ultraviolet B light in *Staphylococcus aureus*

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## ABSTRACT

**Objectives:** *Staphylococcus aureus* is a gram positive bacterium. In recent years, the incidence of *Staphylococcus aureus* skin and soft tissue infections has been steadily increased around the world. Nowadays, the phototherapy is a suitable alternative as a cheap and effective treatment and in some cases can be used in parallel with chemotherapy. That is why, in this survey, we tried to detect the eventual apoptosis feature in UVB-irradiated colonies of *Staphylococcus aureus*.

**Materials and Methods:** The bacterial sample was harvested from the microbial collection center of Islamic Azad University, Shahr-e-Qods branch, Microbiology laboratory. The colonies of *Staphylococcus aureus* were radiated by UVB beam and then, the DNA molecules belonging to control and irradiated colonies were extracted by DNP kit. Next after DNA extraction, the DNA molecules mixed in loading dye were run in 1% agarose gel electrophoresis. Following the electrophoresis, the UV transilluminator was used to observe the orange luminescent DNA bands formed in agarose gel.

**Outcomes:** As it was indicated by experimental practices in the present investigation, no abnormalities, neither DNA laddering bands (apoptosis) nor smears (necrosis), were detected.

**Conclusions:** According to the results showing the lack of DNA denaturation after UVB light exposure, a hypothesis could be advanced on the role of the Heat shock proteins and in particular, HSP70 which would act as an anti-apoptotic mechanism inhibiting the induction of apoptosis in UVB light-exposed colonies of *Staphylococcus aureus*.

**Keywords:** *Staphylococcus aureus*, Apoptosis, Heat-Shock Proteins, Electrophoresis

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## OBJECTIVES

**S***taphylococcus aureus* is a member of humans' normal flora isolating from nasal cavity, skin etc. On the other hand, *Staphylococcus aureus* could be involved in a wide range of suppurative infections associated with skin and soft tissues; e.g. nosocomial infections of surgical wounds. Today, an important percent of the community associated staphylococcal infections like skin infections, are caused by methicillin resistant *Staphylococcus aureus* (MRSA). MRSA is a significant pathogenic agent causing nosocomial infections leading to a high range of morbidity and mortality around the world; because it is resistant to beta-lactams including oxacillin, amoxicillin, penicillin, cephalosporins etc.; hence the infections caused by MRSA must be treated by vancomycin or other medical drugs depending on antibiotic susceptibility (1-6).

That is why, the UVB phototherapy could be an alternate choice in parallel with chemotherapy. Until now, several investigations have evaluated the effects of UVB on *Staphylococcus aureus*. In this research we tried to detect apoptosis feature in the irradiated colonies of *Staphylococcus aureus* through a determined protocol. □

## MATERIALS AND METHODS

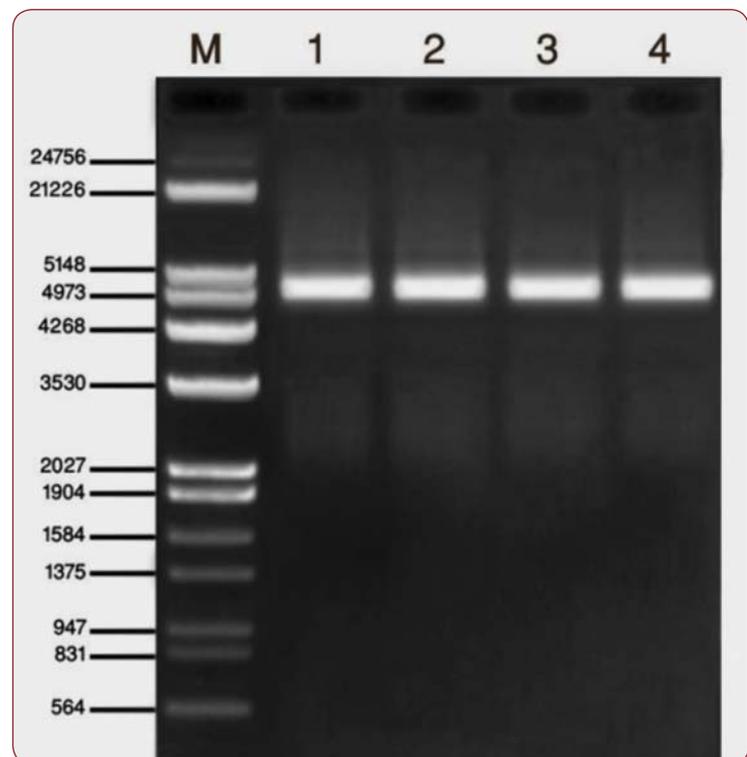
**S***taphylococcus aureus* was isolated from the microbial collection center of Islamic Azad University, Shahr-e-Qods branch, Microbiology laboratory. The bacterial samples were identified through microscopy, Gram staining and biochemical tests as standard traditional diagnostic techniques (7,8). Also, according to our examination, the applied sample of *Staphylococcus aureus* was recognized as bound coagulase or cell associated clumping factor negative strain by slide coagulase test (9,10). *Staphylococcus aureus* was inoculated into four plates containing Nutrient Agar (Merck KGaA, Darmstadt, Germany). Then, the agar plate lawns of *Staphylococcus aureus* were incubated for 72 hours at 37°C. By observing the colonies with significant growth, a plate was chosen as control and the other plates were radiated by UV-transilluminator (Upland, CA, U.S.A.). The colonies were irradiated *in vitro* with UVB beam in the wavelength of 302 nm at a fixed intensity and distance of 8 centimeters for 10 min-

utes above the colonies. Next, the irradiated colonies were incubated within a dark chamber respectively for 1, 24 and 72 hours (7,8,11).

Then, the total genomic DNA belonging to irradiated colonies of *Staphylococcus aureus* as well as control colonies was harvested with the DNP kit (50T, CinnaGen Inc.) according to manufacturer's guidecatalog (7). Finally, the DNA lanes were analyzed after running DNA molecules on an ethidium bromide-stained 1% agarose gel electrophoresis (7,8,11,12).

For comparing the DNA bands in this investigation, DNA weight marker III of CinnaGen Company was used as a molecular weight size marker (Figure 1). The concentration of RNA molecules was low and no obscurity was seen during DNA harvesting (7,13) (Figure 1).

The bands of DNA molecules isolated from control and irradiated colonies of *Staphylococ-*



**FIGURE 4.** The DNA lanes belonging to harvested DNA molecules from control and UVB-irradiated colonies of *Staphylococcus aureus*, running upon 1% agarose gel.

Lane M: DNA weight marker III of CinnaGen Company (Size Marker). It shows the molecule weight of DNA around 5 kbp.

Lane 1: DNA bands related to control colonies of *Staphylococcus aureus*.

Lane 2: DNA bands related to 10-minute-irradiated *Staphylococcus aureus* colonies, kept for 1 hour in a dark chamber after UVB exposure.

Lane 3: DNA bands related to 10-minute-irradiated *Staphylococcus aureus* colonies, kept for 24 hours in a dark chamber after UVB exposure.

Lane 4: DNA bands related to 10-minute-irradiated *Staphylococcus aureus* colonies, kept for 72 hours in a dark chamber after UVB exposure.

*cus aureus* were observed to evaluate the eventual apoptotic patterns of DNA Laddering bands (7,8,11). □

### OUTCOMES

The harvested DNA molecules of control and 10-minute-UVB radiated colonies belonging to *Staphylococcus aureus* were run upon 1% agarose gel, although neither smears (necrosis) nor DNA laddering (apoptosis) bands were detected. As the figure 1 shows, no abnormality or uncertain and doubtful pattern was observed in DNA lanes. As a practical evidence, the procedures was repeated thrice in detail. □

### DISCUSSION

The progressive resistance of pathogenic bacteria including *Staphylococcus aureus* to multiple antimicrobial treatments has increased the application of phototherapeutic treatment for skin disorders (14-16). Based on wavelength, the UV beam is divided into three groups including UVA (320 nm to 400 nm), UVB (290 nm to 320 nm) and UVC (200 nm to 290). Usually, the UVA and UVB are used as phototherapeutic means to treat skin disorders and UVC is used in wound healing (11, 17). Furthermore, UVB beam is able to trigger apoptosis reactions in the both domains of cells; prokaryotes and eukaryotes (18-20).

The long-term irradiation of UVB beam causes epidermal damage and skin cancer in humans, inducing apoptosis and DNA damages, however, the use of short-term UVB phototherapy is noted as a cheap and effective way to treat the bacterial skin infections (21-24). There are different investigations that confirm the deadly effect of UVB light on *Staphylococcus aureus* (25-27).

According to different studies, UV light including UVB ray may trigger a group of stress proteins which are called Heat shock proteins (HSPs). There are a wide range of HSPs in prokaryotic and eukaryotic cells which are responsible for cells protection. The expression of cellular HSPs and in particular HSP70 is up-regulated (28-30).

According to our *in vitro* experimental survey, a period of 10-minutes UVB radiation with the wavelength of 302 nm at a fixed distance of 8 centimeters was proposed to stimulate the feature of apoptosis (7,8,11,28).

The UVB beam constitutes cyclobutane pyrimidine dimers and 8-hydroxy-2'-deoxyguanosine, compounds which induce apoptosis feature in cells, and providing DNA damages. Hence, the prokaryotic and eukaryotic cells possess diverse repair systems such as dark repair and photo reactivation. So, in the present research, the irradiated colonies of *Staphylococcus aureus* were incubated in dark condition (7,8,11,19,28,31-33).

As it was mentioned in the Results section, no abnormality; neither smear (necrosis) nor laddering band (apoptosis) was observed in DNA molecules isolated from 10-minutes exposed colonies of *Staphylococcus aureus* that were run upon 1% agarose gel electrophoresis. According to investigation which has been done by Minoru Matsuda et al. (24) and what we have got in our study, it seems that, UVB beam is an important inducer of Heat shock proteins, and in particular, HSP70; The protein of HSP70, stimulates base excision repair and suppresses DNA damages through its anti-apoptotic properties. May be, the expression of HSP70 protects cells from UVB-inducing apoptosis (24).

More studies in this field could provide a successful UV radiation protocol to gain an acceptable UV therapeutic medication by inducing apoptosis in bacterial cells of *Staphylococcus aureus*. □

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### CONFLICT OF INTEREST STATEMENT

There is no conflict of interest.

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