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# A Comparative study of the laser beam effect on some virulence factors of Staphylococcus *aureus* isolated from different sites of the human body

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**Abstract**:

Staphylococcus aureus has been subjected to low-level laser radiation at various exposure periods. From January 2019 to September 2019, 140 skin and UTI samples (swabs) were obtained from patients at Medical City and Medical City Teaching Hospital who had burns, wounds, and UTI s infections. 50 isolates of S. aureus were identified through cultural traits, microscopic examination, biochemical tests, API staph, and the Vitek system. The 15 isolates were subjected to a diode laser at an exposure length of 1 minute, 3 minutes, and 5 minutes. Before and after irradiation, it was evaluated how active the bacteria were in producing alpha toxin and how sensitive they were to antibiotics. The results of the alpha toxin generation of 15 irradiated isolates showed that the amount of toxin produced decreased at different irradiation exposure times [1, 3, and 5 min.]. The diode laser's impact on S. aureus' sensitivity to antibiotics results in a small increase in the diameter of the drugs' inhibition zone at various exposure times.

**Keywords:** *S.aureus*, laser beam, virulence factor, burn, wound, UTI, an antibiotic.

## 1. Introduction

Staphylococcus aureus is a notorious and widespread bacterial pathogen that causes hundreds of thousands to millions of more severe, invasive infections annually as well as an undetermined number of straightforward skin infections [1]. While most S. aureus infections do not pose a life-threatening danger, they can still result in significant morbidity and suffering. Furuncles, abscesses, and wound infections, which are relatively severe skin infections, are among them. They pose a major public health burden due to their prevalence (several million each year in the U.S.) [2]. MRSA infections have greater mortality, morbidity, and hospitalization rates than infections caused by methicillin-sensitive S. aureus (MSSA) [3].

Staphylococcus aureus shockingly accounts for 70% of all UTI infections worldwide [4]. These could result in morbidity, loss of vision, and tissue injury [5]. S. aureus infection is the most common cause of microbial keratitis (MK) in Australia and the USA [6, 7]. The corneal illness known as MK can be dangerous to one's vision. Additionally, S. aureus can cause non-infectious corneal infiltrative events (niCIE) and conjunctivitis while wearing contact lenses [8]. S. aureus is well known for encoding a broad variety of virulence factors, which allow it to cause a variety of diseases. The genetic makeup of S.

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aureus strains affects their virulence and the pathogenicity related to their illness. [9].

The ability of S. aureus to produce a variety of extracellular and surface virulence factors with sticky properties for a variety of molecules adds to its ability to cause a variety of infections and toxicity. (MSCRAMMs). The extracellular products include toxins with superantigenic properties, including the exfoliative toxins A-B, toxic shock syndrome toxin-1, enterotoxins A-E, G-K, M-O, and Q, as well as other compounds, like Panton-Valentine leukocidin [10]. One more pathogenicity and resilience tactic is the development of biofilms. This technique relies on the production of extracellular polysaccharide compounds, which cause bacterial cells to congregate as multilayer biofilms and avoid the immune system's and antibiotics' side effects [11].

Damaging chance seeker Because it can create biofilms on the tissues and implants of its hosts, Staphylococcus aureus is a common cause of nosocomial infections. Consequentially, chronic infections are frequently produced, including those that arise during catheterization or in individuals with cystic fibrosis, osteomyelitis, or endocarditis [12]. Typically, biofilm formation can be broken down into three stages: primary surface attachment, maturation, and biofilm dispersal [13]. The issue with treating and controlling the disease stems from both the prevalence of S. aureus and its quickly rising antibiotic resistance. Because of the interaction of these variables, S. aureus is one of the most prevalent nosocomial and community-acquired infection-causing bacteria [14].

Since its conception in 1960, laser technology has taken up a significant amount of space in science and technology. Numerous studies in the domains of physics, biology, chemistry, electronics, and other sciences have used laser technology. Laser technology has caused many significant developments in the progress of research. A lot of challenges in the field of microbiology have been solved using laser technology in recent years. The most prevalent pathogens are S. aureus, which is widely distributed in all habitats. In numerous human bodily systems, they induce numerous disorders [15].

One of the most significant virulence aspects is the bacterial ability to manufacture various enzymes and toxins that play a part in invasion and tissue damage like hemolysin toxin. This ability allows the bacteria to bypass most of the body's defense mechanisms and subsequently cause infection. Additionally, bacteria are capable of resisting a wide range of antibiotics, which has become a widespread issue worldwide. Antibiotic resistance is linked to a variety of processes, including changes to the target site, efflux system, metabolic pathways, or production of modifying enzymes. One of the most significant modifying enzymes is -lactamase, which can kill bacteria and eliminate the most significant class of medicines, known as -lactam antibiotics, which include penicillins and cephalosporins [16]

## 2. Material and method

### 2.1. Isolation and identification of bacteria

From January 2019 through September 2019, 140 skin and UTI samples (swabs) were taken from patients at Medical City and Medical City Teaching Hospital who had keratitis, burns, or open wound infections. Identification of these isolates was also done using the Gram stain, API staph [Biomerieux], and Vitek system as well as the form, color, size, edges, and height of the colony on the surface of brain heart infusion (Himedia) and blood agar plates [17]. These isolates were also identified using a variety of procedures, such as the catalase test, the mannitol fermentation test, the coagulase test, the alpha-toxin test, and the hemolysis patterns on blood agar [18].

### 2.2. Alpha hemolysin assay (Micro titer plate method)

Before laser exposure, an experiment was conducted to check for the presence of 50 isolates of alpha-hemolysin toxin in S. aureus supernatants: At 37 C, bacteria were grown for 18 hours in Tryptone soy broth. Each isolate's supernatant was transferred to sterile test tubes following a 15-minute centrifugation at 7000 rpm. By adding 0.1 ml of 2% washed rabbit red blood cells and 0.1 ml of duplicate bacterial supernatant to the well of a U-shaped

microtiter plate, you may determine whether or not an isolate is capable of producing alpha-hemolysin. The negative control (0.1 ml of normal saline + 0.1 ml of cleaned red blood cells) is shown in the first and second wells of each horizontal line. The microtiter plate was incubated for one hour at 37° C. After incubation, plates were held at 4° C for 2 to 18 hours, after which the RBC lysis result was determined [19].

### 2.3. Antibiotic sensitivity

The Kirby-Bauer disc diffusion experiment was used to test the sensitivity of S. aureus isolates [20]. Ciprofloxacin (CIP (5 g), chloramphenicol (C(30 g), Gentamicin (CN(10 g), Nitrofurantoin (F(300 g)) and other antibiotic discs were employed in this research and used vitek system that performed the results.

### 2.4 Laser system

The CW diode laser (Eltech S.R., Italy) produced laser light at a wavelength of 805 nm, IR light (light in the infrared region of the spectrum, which ranges from 700 to 2000 nm), and a thin, flexible glass fiber with a width of 8 mm [21]. The output power was 0.94, 2.01, and 2.766 W, and the exposure time was 1 s. (1 min.). Power concentrations were 1.87, 4.0, and 5.49 W/cm2 for 0.94, 2.01, and 2.76 W, respectively.

Irradiation technique: From the brain heart infusion agar slant, a loopful of the culture was transferred to a test tube containing brain heart infusion broth, where it was cultured at 37°C for the next day. The precipitate was resuspended in physiological saline after being centrifuged at 3500 rpm for 10 minutes, and the supernatant was discarded. To achieve a homogeneous suspension that could be compared to the McFarland solUTIon (1.5*108 CFU/ml), a vortex mixer was used [22].

One Eppendroff tube containing one milliliter of the suspension from each species of diluted bacteria was sterilized, and one milliliter of the suspension was then exposed to laser light at various exposure times; the other Eppendroff tube served as a control. Both the laser-irradiated and control suspensions were then spread out on brain heart infusion agar and incubated at 37°C for an overnight period before being evaluated for biochemical traits and their sensitivity to antibiotics [23].

## 3. Results and discussion

Fifty isolates (35.7%) of all the isolates from skin and UTI swabs were S. aureus. These isolates tested positive for catalase, coagulase, hemolysin production (alpha-toxin), and the ability to ferment mannitol, according to the results. The data provided by [6,7] and morphological and biochemical characterization were consistent. In addition to the tests mentioned above, the API-staph and Vitek systems also perform biochemical identification, which validated the earlier traditional identification.

There is no effect of irradiation on the synthesis of the enzymes (Catalase and Coagulase) or the fermentation of mannitol in just 15 isolates of S.aureus that are treated with a diode laser (2w) at various exposure durations. When S. aureus isolates were exposed to a photosensitizer under the same conditions, beta hemolysis activity decreased, but alpha-hemolysin production showed a 40% reduction (in toxin production) after 5 minutes of exposure (the results show a significant decrease in hemolysin production compared to control) [25].

According to Tubby et al. (2009), laser light alone had no discernible effect on the activity and generation of hemolysins of S. aureus isolates. Instead, after receiving a small dose while being around methylene blue, the hemolysin's activity was completely diminished.

These results indicate that of the tested virulence factors, alpha-hemolysin is the most susceptible, potentially as a result of the nature of its amino acid composition, which may make it more susceptible to attack by reactive oxygen species. Several distinct cell types can be lysed by the S. aureus alpha-hemolysin toxin, which damages membranes. The interruption of ion transport across host cell membranes caused by alpha-hemolysin is expected to have a multitude of negative effects on host cells, ultimately causing apoptotic cell death and edema [26].

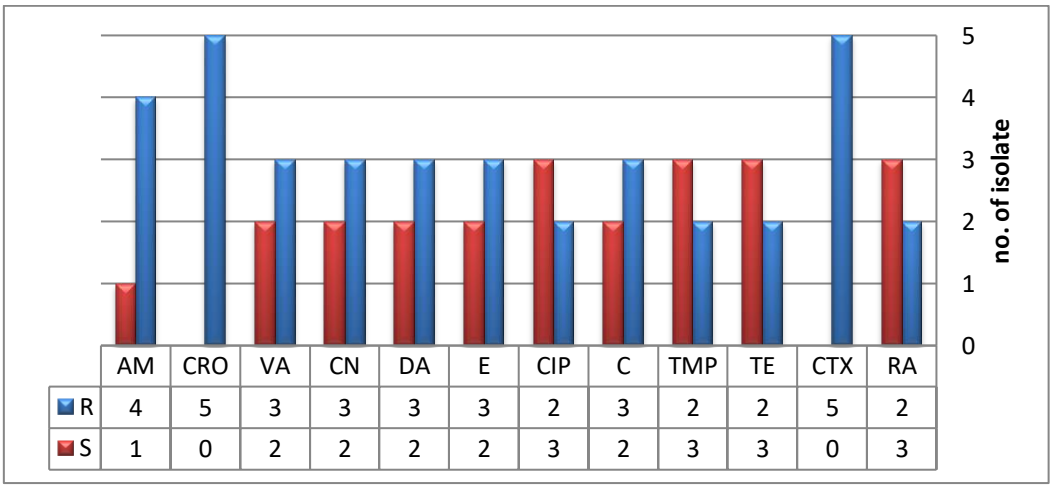
Numerous infection models, including mastitis and pneumonia, have shown the contribUTIon of alpha-

hemolysin to the pathogenicity of S. aureus. Alpha-haemolysin also has immune-modulating qualities, including the capacity to cause the production of cytokines that promote inflammation [27]. As a result, photodynamic therapy that activates alpha-hemolysin may help to both get rid of infectious organisms and guard against damaging inflammatory processes.

Several antibiotics, including chloramphenicol (C), ciprofloxacin (CIP), gentamicin (CN), and nitrofuran (F), and other antibiotic were tested for susceptibility using the disc diffusion method to S. aureus isolates. following several (1,3,5) min exposures to a diode laser as seen in Figure (1,2,3,4).

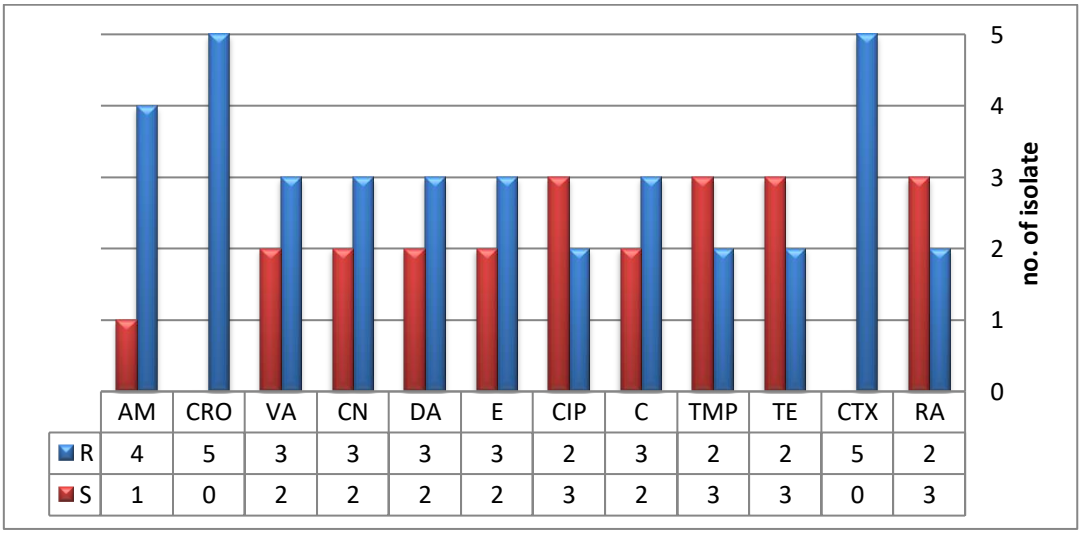
Results indicate that the majority of isolates become more or less susceptible to antibiotics at various times after 1,2,3,4 min exposure [25].

The result of the researcher resembles increased susceptibility of S. aureus isolates to Ciprofloxacin and Nitrofuranon after one minute of laser exposure, while the same isolates exhibit increased susceptibility to Gentamicin and Chloramphenicol after three to five minutes of laser exposure (antibiotic sensitivity is negligible) [25].



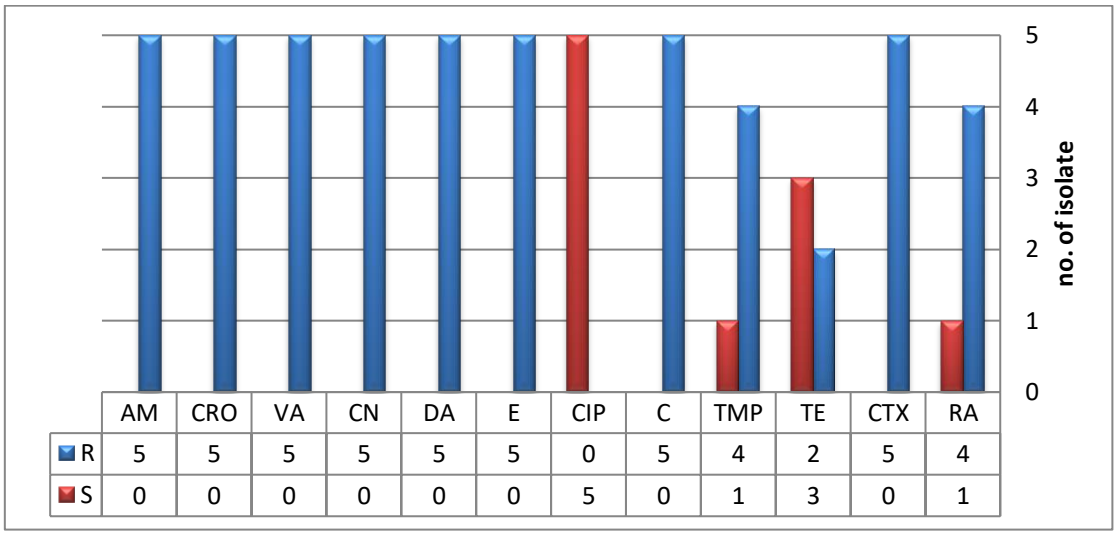

| | AM | CRO | VA | CN | DA | E | CIP | C | TMP | TE | CTX | RA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | 4 | 5 | 3 | 3 | 3 | 3 | 2 | 3 | 2 | 2 | 5 | 2 |
| S | 1 | 0 | 2 | 2 | 2 | 2 | 3 | 2 | 3 | 3 | 0 | 3 |

Figure (1): Shows the sensitivity and resistance of S. aureus isolate before exposure to laser radiation.



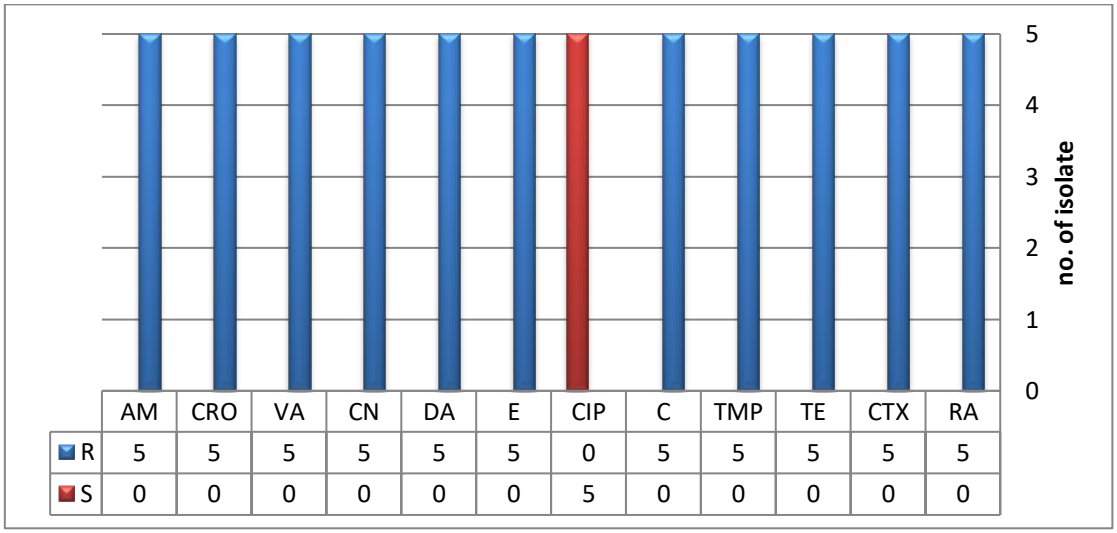

| | AM | CRO | VA | CN | DA | E | CIP | C | TMP | TE | CTX | RA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | 4 | 5 | 3 | 3 | 3 | 3 | 2 | 3 | 2 | 2 | 5 | 2 |
| S | 1 | 0 | 2 | 2 | 2 | 2 | 3 | 2 | 3 | 3 | 0 | 3 |

Figure (2): Shows the sensitivity and resistance of S. aureus isolate after exposure to laser radiation in (1 min).




| | AM | CRO | VA | CN | DA | E | CIP | C | TMP | TE | CTX | RA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | 5 | 5 | 5 | 5 | 5 | 5 | 0 | 5 | 4 | 2 | 5 | 4 |
| S | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 1 | 3 | 0 | 1 |

**Figure (3): Shows the sensitivity and resistance of S. aureus isolate after exposure to laser radiation in (3 min).**




| | AM | CRO | VA | CN | DA | E | CIP | C | TMP | TE | CTX | RA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | 5 | 5 | 5 | 5 | 5 | 5 | 0 | 5 | 5 | 5 | 5 | 5 |
| S | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |

**Figure (4): Shows the sensitivity and resistance of S. aureus isolate after exposure to laser radiation in (5 min).**

These results were in agreement with those of [28, 29], who discovered that diode laser irradiation increases bacteria's susceptibility to antibiotics over time and at increasing doses. As opposed to another study that found no relationship between laser light and S. aureus's sensitivity to antibiotics [30] these findings conflict with that study.

Changes in the susceptibility of bacterial isolates to antimicrobial agents following treatment with a diode laser may be the result of the laser and antimicrobial agent working together to increase the sensitivity of the bacterial cell to these drugs.

Additionally, these adjustments in bacterial pumping systems, or efflux pumps, which are primarily in charge of bacterial resistance to or sensitivity to antibiotics, may be the cause of these modifications in bacterial sensitivity. The susceptibility of bacteria to antibiotics may have risen due to the bacteria's inability to manufacture certain enzymes that chemically modify certain drugs [31].

Antibiotics (ciprofloxacin, oxacillin) were more effective against MK strains from Australia than those from the USA. There is a lot of research on the potential virulence factors that S. aureus may use to cause keratitis, but there are considerably less data on

how these variables may contribute to conjunctivitis or niCIE [32].

Using the well-known whole genome sequencing (WGS) technique, virulence factors, novel bacterial lineages, and their population structures can all be discovered [33]. In genome-wide association studies and comparative genomics, clinical isolates can be used to find genetic factors that might be important in the setting of particular infections. For instance, WGS has been used to effectively study S. aureus isolates from systemic infections (bloodstream, airways, endocarditis, and joint infections) to better understand specific population structures and explore the relationship between virulence factors and patient outcomes [34].

In addition to revealing high levels of diversity and the co-presence of local, global, livestock-associated, and hypervirulent clones, WGS of S. aureus strains isolated from different diseases (airways, soft tissues, and skin lesions) also showed that several virulence factors and clones were disease-specific. For instance, the sequence type ST5 was linked to enterotoxins (SE), and ST22 was linked to the toxin that triggers toxic shock syndrome, TSST-1 [35].

Another research looked at the impact of laser therapy on commensal nasal isolates and isolates from infections in prosthetic joints. It was found that the commensals shared a clonal complex (CC) and that there was a nearly equal prevalence of virulence genes in isolates from commensal and prosthetic joint infections in patients with arthroplasty, suggesting that commensal S. aureus nasal clones can infect joints [34].

## 4. Conclusion

Numerous research largely concentrated on illuminating specific molecular elements that are essential throughout these S. aureus biofilm formation phases. As a result, both proteinaceous and non-proteinaceous components have been described as playing significant roles in adhesion to abiotic surfaces and host cells, as well as in the integrity and structure of biofilms.

Certain S. aureus isolates developed increased sensitivity to antibiotics and lost the ability to create beta-hemolysis and alpha-toxin while, the production of mannitol fermentation, coagulase, and catalase was unaffected by laser irradiation.

**Conflict of interest:**

There are no conflicts of interest.

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