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Antibacterial Effect of Low-Level Laser (Diode 405 nm) on Antibiotic-Resistant Enterococci Clinical Isolates (*In Vitro*)

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Abstract

Background: *Enterococcus* is a part of normal gastrointestinal flora in human body. Nevertheless, antibiotic-resistant *Enterococcus* (ARE) is considered a key factor in nosocomial infections which result in a considerable increase in the rate of patient death due to referring of numerous patients to health centers annually, or lead to extended disease convalescence.

Objective: This study aimed to evaluate the bactericidal effect at 405nm diode at a laser power of 30 mW on ARE viability of clinical infections.

Materials and Methods: In the present study, 30 isolates underwent antibiotic susceptibility test (AST) in which sensitivity to piperacillin (100 µg), rifampin (5 µg), and oxacillin (1 µg) were measured based on the Clinical and Laboratory Standards Institute (CLSI) guidelines. Afterwards, ten most resistant isolates were selected and irradiated by a 405 nm diode laser at a power of 30 mW for 180 and 240 seconds. The data were reported statistically as mean ± standard deviation, and the analysis of the data on varied bacteria was performed using ANOVA. The result was evaluated by SPSS software and *P* value ≤0.05 was interpreted to be significant.

Results: Bacterial viability decreased unsteadily to 10 resistant isolates. Moreover, enhancing irradiation time caused a lower viability rate in such a way that the viability of isolate 9 having the lowest viability rate was reduced from 2.94% in 180 seconds to 0.58% in 240 seconds. The result was evaluated by SPSS software and *P* value was determined to be significant, and *P*≤0.05 was laser irradiation for either 180 s or 240 s.

Conclusion: Following the study results, 405 nm diode laser could be applied as a tool for eliminating clinical ARE, and it was useful for preventing hospital-acquired infections.

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Background

Enterococcus is a gram-positive, catalase-negative bacterium. *Enterococcus faecalis* and *Enterococcus faecium* are prominent species that symbiotically exist in the human intestine and are part of the gastrointestinal flora. However, antibiotic-resistant enterococci causes widespread infections such as nosocomial infections under certain circumstances.[1] In the beginning, these bacteria used to be considered insignificant but during the last decades, inappropriate antibiotic over-prescription has led to the dominancy of antibiotic-resistant strains which resulted in some negative consequences including unexpected increase in pathogens activities,[1,2] significant decrease in bacterial sensitivity to a variety of medications, lack of proper patient response to antibiotic treatments, and developing of the side effects.[3] The appearance of multidrug-resistant strains in several regions of the world is due to the genetical alterations in antibiotic-resistant genes like mutation, acquisition of external genomes such as a plasmid, transposon, mobile genetic elements, and other transferring methods.[4] Antibiotic-resistant genes can be easily exchanged between Enterococcus bacteria. Furthermore, microorganisms are highly capable of transmitting between patients and the environment either

directly or indirectly, and are also capable of tolerating unfavorable situation for a long time. These elements are found in the widely stable prevalence of the strains in different situations.[5,6] These characteristics have led to the deficiency of common popular chemical methods for bacterial disinfection. Hence, awareness of active methods for controlling these kinds of microorganisms is of considerable importance.

In recent years, with the global growth of laser application specifically in biology and medicine, studying radiation effects on cells and different organisms have been taken into consideration.[7] Klinke et al studied Nd: YAG laser effects on root canal infection caused by Streptococcus mutants in teeth for the first time. A study conducted in 2002 examined an 810 nm diode laser effect and found a significant decrease (74%) in the root canal dentin bacterial population.[8] Gutknecht et al proved that the bactericidal effect of 980nm diode laser could eliminate bacteria existing in a deep part of the root canal wall dentin of a cow which led to endodermic treatment progress.[9] With the expansion of diode laser application as a cheap and available laser source, numerous researches have been performed into Enterococci undergoing laser irradiation. Asnaashari et al and Tokuc and colleagues' findings illustrated significant bactericidal effects on Enterococci existing in the bacterized tooth root canal.[10,11] Most researches have focused on oral contamination caused by *Enterococcus* thus far and, therefore, the effects of various wavelengths on enterococci contamination – especially AR strains, have received little attention. This study aimed to evaluate 405 nm diode laser bactericidal effects on AR enterococci viability at a power of 30 mW for 3 and 4 minutes to assess the capability of the method to quickly eradicate existing infections in the hospitals responsible for high death rate. In this study, first, AR strains were isolated by completing some antibiogram tests, and then the remaining bacteria left after laser irradiation were examined.

Materials and Methods

Clinical Sample Collection

To provide clinical antibiotic-resistant isolates, the specimens were collected from samples of hospitalized patients. Clinical isolates were identified by phenotypic tests and biochemical properties.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing (AST) was performed by the disk diffusion method based on the Clinical Laboratory Standard Institute (CLSI 2017) guidelines.

To perform the antibiogram tests, the bacterial isolates were initially inoculated by special culture media for the antibiogram tests in the MHA medium (Merck, Germany). Then piperacillin (100 µg), rifampin (5 µg), and oxacillin (1 µg) antibiotic disks (MAST company, UK) were used for susceptibility tests. Afterward, the plates were incubated at 37^{0}C for 18 h and, subsequently, the thickness of the clear zone was measured and reported in mm.

Laser Irradiation Assay

Thirty clinical antibiotic-resistant isolates were selected out of 100 isolates for the laser irradiation tests. To evaluate the sensitivity of the specimens to different diode laser wavelengths, at first, the isolates were cultured, and then a 10 µL suspension equal to McFarland was cultured on a 96-well microplate containing 100 µL MHB medium (Merck, Germany). After culturing the isolates, the laser beam (China) irradiated them at 405 nm and 810 nm, power of 30mW for 180 and 240 seconds, respectively. Wells containing bacteria which underwent no laser irradiation were used as negative controls. Furthermore, wells containing medium were used separately as intrinsic controls to study the lack of medium contamination. To learn about the laser beam antibacterial activity with a specified wavelength, 10 µL was taken from all clinical isolates and cultured individually in the MHA medium. Then the plates were incubated at 37°C for 24 hours and, ultimately, the colonies were counted and compared to the control. Finally, the results were reported and then all the above-mentioned tests were repeated three times.

Statistical Analysis

The data were reported statistically as mean ± standard deviation, and analysis of the data in varied bacteria was studied by ANOVA. The result was evaluated by Prism 6 software and $P \leq 0.05$ was interpreted to be significant.

Results

In this study, 27 out of 30 specimens (90%) were found resistant to piperacillin, rifampin, and oxacillin. One isolate had resistance to piperacillin and rifampin, and two isolates developed resistance to each of the above-mentioned antibiotics (Figure 1).

Twenty out of thirty clinical isolates were sensitive to laser irradiation. Moreover, 10 isolates demonstrated maximum laser efficiency on bacterial viability in 240 seconds as follows, respectively: isolate number 9 (0.58%), isolate number 4 (1.04%), isolate number 7 (3.44), isolate number 6 (4.14), isolate number 8 (7.61%), isolate number 2 (25.8%), isolate number 3 (30.23%), isolate number 10 (41.66%), isolate number 1 (58.18%), and isolate number 5 (71.42%) (Figure 2).

The maximum laser efficiency in 180 seconds observed in isolate number 9 (2.94%), isolate number 8 (4.76%), isolate number 7 (5.6%), isolate number 4 (7.29%), isolate number 6 (7.46%) isolate number 2 (32.25%), isolate number 3(37.20%), isolate number 10 (62.5%) and isolate number 1 (72.71%), respectively. However, there was an increase in the colony number of isolate number 5 (142.8% viability) (Figure 3).

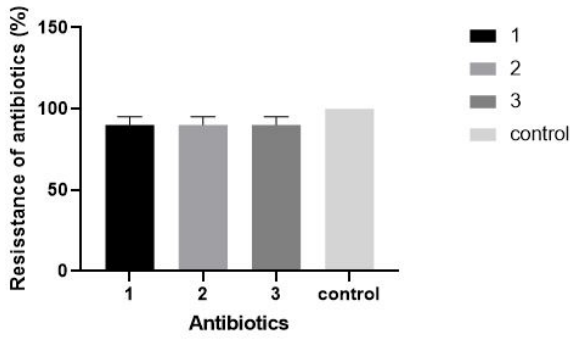

Figure 1. The Antibiotic-Resistance Percentage of 30 Antibiotic-Resistant Enterococci.

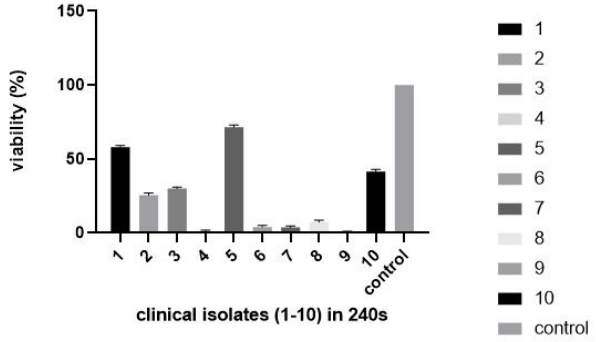

Figure 2. The Viability Rate of Ten Clinical Isolates (1-10) Due to Laser Irradiation With a Wavelength of 450 nm and a Power of 30 mW in 240 Seconds.

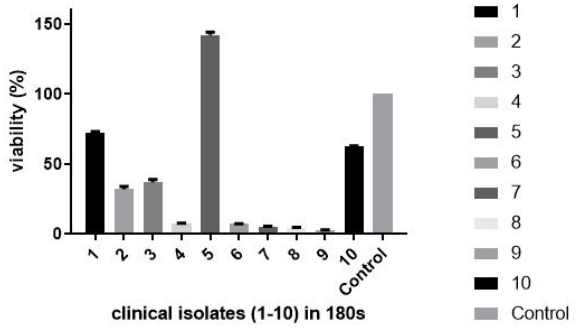

Figure 3. The Viability Rate of 10 Clinical Isolates (1-10) Due to Laser Irradiation With a Wavelength of 450 nm and a Power of 30 mW in 180 Seconds

Discussion

In this study, 405 nm diode laser irradiation at a 30 mW power for 3 and 4 minutes could be adequate for growing clinical antibiotic-resistant isolates of enterococci. Therefore, in most cases, as the irradiation time increased, the number of bacterial colonies decreased after the exposure. However, the increase in the number of colonies in an isolate was merely observed after laser exposure, which could be due to stimulating bacterial deviation cycle through laser irradiation requiring more investigation as a new bacterial behavior. Nowadays, laser application has been developed in different fields. Bactericidal effects of a variety of lasers have been reported in several studies, including the impact of diode laser on bacterial viability. Nevertheless, there are few published studies investigating diode laser application in bacterial infection perpetuity and, therefore, this study can be something of a novelty. Scientific researches have revealed that the current application of chemical sanitizers is no longer sufficient for eliminating bacterial infection. In1900, photodynamic therapy (PDT) was recognized as a promising disinfecting methods.[10] The novelty of PDT has been examined through several researches in recent years.

Fonseca et al conducted a research that detected PDT elicits microbial cells.[12,13] Kline et al studied the impact of Nd: YAG laser on root canal infection caused by streptococcus mutants. Comparing the irradiated specimen and the control showed a significant bacterial population decrease resulted from the antibacterial effect of laser irradiation.[8] Asnaashari et al reported that the photodynamic effect of 810 nm laser was stronger than a 630 nm LED lamp on *E. faecalis* living in human teeth.[11] Garcez et al indicated that there was no significant bactericidal effect while applying NaoCl or laser separately.[14] On the other hand, using NaoCl and 685 nm laser simultaneously resulted in a reduced amount of *E. faecalis* in the teeth root canal. This suggested that the application of laser was more effective than NaoCL in endo-dental treatments.[14] Gutknecht et al found that the bactericidal effect of 980 nm diode laser on bacteria living in cow teeth root canals had an impact on development of Endo-dental treatments.[9] Schoop et al proved that all diode laser wavelengths were suitable to disinfect deep layers of teeth.[15] Topaloglu et al suggested that using 809 nm laser with ICG (indocyanine green) produced significant bactericidal effects due to the elimination of antibiotic-resistance in gram-positive or gram-negative bacteria.[16] Gutknecht et al demonstrated that applying Cr: Er,Cr:YSGG (at 2780 nm wavelength, power of 1.06 W, 50 Hz frequency) and diode laser (at 940nm wavelength, power of 0.51 W) simultaneously could be remarkably effective in eradicating E. faecalis existing in 100 µm-deep of dentin.[17] The results of the above-mentioned research studies are consistent with our data. Tokuc et al studied the effect of 2780 nm Er. Cr: YSGG accompanied by 940 nm diode laser on Enterococcus at the same time and conclusively proved the effectiveness of the experiment in eliminating *E. faecalis*.[10] A study carried out in 2000 on the 810 nm diode laser effect indicated a 74% drop in the bacterial population existing in the root canal on average.[18] Ghanbari et al findings revealed bactericidal effects of diode laser (from 440 nm to 480 nm) at 830 nm with 22 µm Erythrosine for 1-5 seconds on *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, and it emerged that the more laser irradiation time was spent, the more antibacterial effects were produced due to exposure. In our

study compared to Ghanbari and colleagues, more effects were detected by applying a 405 nm diode laser in 3 and 4 minutes. However, the study results were concordant in terms of the dependence of antibiotic efficiency on time.[19] Besides, Klinle et al reported the highest antibacterial effect (92.9%) by investigating the effect of 980 nm Nd: YAG, power of 1.5 W, and frequency in the order of 15Hz on *Streptococcus mutants* existing in 300 µm deep of the teeth root canal.[8] Gutknecht et al concluded that the most antibacterial effect on *E. faecalis* in 100 µm-deep of dentine could be achieved by applying a 980 nm laser at a power of 2.8 W. Moreover, bacterial death was estimated at 97%.[9] Andres et al examined periodontal pocket microbes. In their study, both microbial specimen and the control-both containing H2O2- were irradiated by diode laser. Evaluating the samples after 6 months demonstrated the great impact of diode laser on the bacterial population reduction. Moreover, they stated that the laser was effective on decreasing inflammation as well.[20] In our study, only one strain was examined; while, in their study, different bacterial strains including *Actinobacillus*, *Actinomycetemcomitans*, *Prevote*, and *Porphyromonas gingivalis* were investigated. The results of both studies, however, were consistent.

In another study by Chan and Lai, the effects of different laser wavelengths on periodontal microbes in PDT were explored. In their study, *Actinobacillus actinomycetemcomitans*, *Fusobacterium* nucleatum, *Porphyromonas* gingivalis, *Prevotella intermedia*, and *Streptococcus sanguis* mediums were irradiated by He-Ne laser (632.8 nm, 30 mW) and diode laser (665 nm, 100 mW) (830 nm, 100mW) in presence and absence of methylene blue as a photoinitiator. While the control was only exposed to methylene blue, the results showed that the 100 mW laser accompanied by methylene blue had the inhibitory effect on colonization and bacterial growth in 1 second in comparison with the above-mentioned control group.[21] These findings were consistent with data; the only difference was the absence of methylene blue as a photoinitiator. Following the results of a study by Fontana et al, 810 nm 400-1200 mW diode laser had antibacterial properties on periodontal pockets bacterial specimens which suggested the same effect on bacteria in different wavelengths and bacterial strains.[22] Beer et al indicated that 830 nm and 940 nm diode laser could be effective in decreasing *E. coli* and *E. faecalis* population in human teeth root canals. Considering the differences among the types of the studied bacteria, the results of Beer and colleagues' study proved to be similar to our study results.[23]

Conclusion

Our study results indicated that the irradiation of 405nm diode laser at a power of 30 mW for 3 and 4 minutes could restrain the growth of clinical antibiotic-resistant isolates. It was noticed that as more laser irradiation time was spent, less bacterial viability was achieved. Therefore, this method has a considerable potential for eliminating clinical antibiotic-resistant enterococcal strains.

Authors' Contributions

RKH: performing laboratory operations and writing original draft; NS: Conceptualization, methodology, Supervision, validation; AK: methodology, validation; RM: Supervision, validation; PA: reviewing, and editing; MRP: Methodology

Ethical Approval

This research was conducted in accordance with the protocol approved by the Shahid Beheshti University, Faculty of Biological sciences and technology, Iran.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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