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# Protective Effects of Laser-irradiated *Streptococcus pneumoniae*: A Novel Vaccine Candidate

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## ABSTRACT

**Background:** *Streptococcus pneumoniae* is a significant cause of community-acquired pneumonia worldwide. Pneumococcal vaccines can prevent severe disease; however, their high cost, limited serotype coverage, and variable effectiveness across populations underscore the need for alternative vaccine strategies. Laser irradiation has been proposed as a novel approach to attenuate bacterial virulence while preserving immunogenicity.

**Materials and Methods:** Clinical isolates of *S. pneumoniae* were irradiated using low-energy laser irradiation at wavelengths of 660 nm, 820 nm, and 915 nm to prepare an inactivated bacterial vaccine. Sterility testing confirmed complete bacterial inactivation. BALB/c mice were immunized three times at two-week intervals and were then challenged with wild-type *S. pneumoniae*. A commercial pneumococcal vaccine served as a positive control. Antibody responses (immunoglobulin M [IgM], IgG, and IgA) were measured, and survival was monitored for 21 days post-challenge.

**Results:** Irradiation at 915 nm was the most effective for bacterial inactivation. Mice immunized with the 915 nm-inactivated vaccine showed significantly higher levels of IgM, IgG, and IgA compared to the unvaccinated group. Following bacterial challenge, all mice in this group survived, whereas 40% of mice immunized with the commercial vaccine died within 21 days after challenge.

**Conclusion:** Low-energy laser irradiation at 915 nm effectively inactivated *S. pneumoniae* while retaining its immunogenic properties. Immunization with this preparation elicited robust antibody responses and provided complete protection in mice, suggesting that it may serve as a novel pneumococcal vaccine candidate.

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## Introduction

*Streptococcus pneumoniae* is a gram-positive bacterium with many serotypes [1]. Until recently, more than 100 serotypes have been identified [2]. Pneumococcus is known to be the most common cause of community-acquired pneumonia and the causative agent of diseases, such as otitis media, meningitis, and bacteremia [3, 4]. Pneumococcus colonizes the nasopharynx asymptomatically but is one of the major causes of high mortality and morbidity in infants, the elderly, and immunocompromised persons [1, 4].

Pneumococcal vaccinations may be beneficial for preventing severe pneumococcal disease. Pneumococcal conjugate and polysaccharide vaccines cover more than 20 serotypes of *S. pneumoniae*. Nevertheless, vaccination is commonly recommended only for children and adults aged 65 years or older. Additionally, pneumococcal vaccine may not be widely used because it is not cost-effective for all ages [5]. Due to variations in the effectiveness of vaccines across different societies and the low immunogenicity of polysaccharide-based vaccines, further investigation into whole-cell and protein-based vaccines is warranted [2].

Recent studies have shown that laser irradiation can target bacteria, and these bacteria can absorb different types of laser beams and light, leading to bacterial damage and destruction. Laser therapy is an effective method for reducing infection and may therefore be useful for producing potential vaccines [6, 7].

Inactivated whole-cell vaccines are considered the most convenient and cost-effective options for combating bacterial infections. These vaccines are stable, safe, and can be produced relatively rapidly at low cost. Whole cells are commonly inactivated with heat or formalin treatment [8]. The effectiveness of inactivation methods relies on empirically determined conditions that ensure complete inactivation while retaining immunogenicity [8]. Recently, gamma-irradiation has been considered for the inactivation of *S. pneumoniae* [9, 10]. Therefore, this study aimed to prepare an inactivated vaccine using low-energy laser irradiation and then evaluate antibody levels and assess the vaccine's protective effect in experimental animals.

## Materials and Methods

### Bacterial source

Clinical isolates of *S. pneumoniae* were obtained from patients with respiratory infections at Al-Hussein Teaching Hospital, Samawah, Iraq. Bacteriological identification included sputum culture, microscopic examination, biochemical tests, the API 20 Strep system, and confirmation using the VITEK 2 system [11, 12].

### Laser device

The Omega Diode laser was used in this study. The device includes diode modules that emit wavelengths of 660 nm, 820 nm, and 915 nm used in the study, with output powers ranging from 100 to 400 mW at frequencies of 1 kHz, 5 kHz, and 10 kHz. The exposure time was adjusted to 5, 10, 15, and 20 minutes. One milliliter of each isolate (dilution $1.5\times10^8$ cells/mL) was transferred to a sterile Eppendorf tube. The transferred bacteria were irradiated with a diode laser at room temperature in a dark environment.

### Vaccine preparation

The culture of *S. pneumoniae* was prepared on blood agar. Then, the bacterial suspension in normal saline was vortexed and centrifuged at 6000 rpm for 10 minutes at 4 °C. After twice resuspension with normal saline and preparation of a 0.5 McFarland standard, the suspension was irradiated with the laser to obtain an inactivated vaccine. A sterilization test was performed on the irradiated suspension to confirm the absence of live bacteria in the vaccine. The bacterial suspension was then cultured on blood agar and incubated at 37 °C for 24 hours.

### Immunization program

Fourweekold male BALB/c mice were used to evaluate the vaccine's effectiveness; mice were vaccinated with $1\times10^8$ CFU via the subcutaneous route three times at two-week intervals [13]. Eighty mice were divided into three groups. The first group served as the naive group. It consisted of twenty mice that received no treatment and were kept only for observation. The second group consisted of forty mice, divided into two subgroups, each with twenty mice. Subgroup A was vaccinated with the inactivated bacteria vaccine. In contrast, subgroup B was left unvaccinated. The third group consisted of twenty mice inoculated with the traditional commercial pneumococcal vaccine Prevenar 13 (MOH-Iraq) as a positive control.

### Measurement of specific antibodies

Mice were euthanized, and blood was collected by cardiac puncture. Total antibodies, IgM, IgG, and IgA, in all 80 mice were determined before immunization and ten days after the last vaccination [13] using the COBAS INTEGRA 400 Plus analyzer (Roche-Germany), according to the manufacturer's instructions.

### Survival rate

After the final immunization, mice were challenged with $1\times10^8$ CFU of the virulent strain of *S. pneumoniae* (ST217) via the intranasal route. The survival rate of challenged mice was monitored until 21 days post-challenge. ST217 (serotype 1) has been reported as a major cause of invasive pneumococcal disease worldwide, particularly in low-income countries, where it is associated with invasive pneumococcal disease [14, 15].

### Statistical analysis

Antibody levels were analyzed using student's t-test for comparisons between two groups and one-way analysis of variance (ANOVA) for comparisons among more than two groups. The survival rate was assessed using the log-rank (Mantel-Cox) test. A $P<0.05$ was considered statistically significant. All values were expressed as the Mean±SE.

## Results

### The laser effect on *S. pneumoniae*

A total of five isolates of *S. pneumoniae* were irradiated with a laser. The irradiation results showed a significant decrease in bacterial viability with increasing laser doses, and bacterial survival rates decreased with increasing laser exposure time. No bacterial growth was observed at a wavelength of 915 nm and a frequency of 10 kHz after 15 minutes of irradiation (Table 1). Using the same frequency and exposure time with a wavelength of 820 nm resulted in reduced bacterial growth (Table 2).

A wavelength of 660 nm at a frequency of 10 kHz was used to irradiate the S. pneumonia isolates for different times, and the isolates exhibited different responses. Exposure times from 5-10 minutes produce noticeable changes in the five bacterial isolates, and bacterial

**Table 1.** Determination of the laser lethal time on *S. pneumoniae* using 915 nm wavelength

| Power (w) | Frequency (kHz ) | Time (Minute) | Results |
|---|---|---|---|
| 200 mw 0.2 (w) | 5 | 15 | Reduced growth |
| | 10 | 15 | Reduced growth |
| 300 mw 0.3 (w) | 1 | 15 | Reduced growth |
| | 5 | 10 | Reduced growth |
| | 10 | 10 | Reduced growth |
| | 10 | 15 | No bacterial growth |

**Table 2.** Determination of the laser lethal time on *S. pneumoniae* using 820 nm wavelength

| Power (w) | Frequency (kHz ) | Time (Minute) | Results |
|---|---|---|---|
| 200 mw 0.2 (w) | 5 | 15 | Reduced growth |
| | 10 | 15 | Reduced growth |
| 300 mw 0.3 (w) | 1 | 15 | Reduced growth |
| | 5 | 10 | Reduced growth |
| | 10 | 10 | Reduced growth |
| | 10 | 15 | Reduced growth |

**Table 3.** Effect of laser on *S. pneumoniae* isolates using wavelengths of 660 nm, a frequency of 10 kHz, and a power of 3 W at different time points

| Bacteria Isolates | Minute | | |
|---|---|---|---|
| | 5 | 10 | 20 |
| *S. pneumoniae* 1 | Normal-growth | Normal-growth | Reduced growth |
| *S. pneumoniae* 2 | Normal-growth | Normal-growth | Reduced growth |
| *S. pneumoniae* 3 | Normal-growth | Normal-growth | Reduced growth |
| *S. pneumoniae* 4 | Normal-growth | Normal-growth | Reduced growth |
| *S. pneumoniae* 5 | Normal-growth | Normal-growth | Reduced growth |

growth appeared normal based on colony morphology and colony count, whereas weak bacterial growth and reduced colony numbers were observed after 20 minutes of irradiation (Table 3).

### Survival rate

The survival analysis demonstrated that the SPL vaccine provided complete protection (100% survival) against the bacterial challenge, significantly outperforming both the commercial pneumococcal vaccine and the unvaccinated group. While the commercial vaccine offered partial protection (~55% survival), it was markedly less effective than the SPL formulation. In contrast, all animals in the unvaccinated group succumbed to infection by day 21. These findings highlight the superior protective efficacy of the SPL vaccine candidate (Figure 1).

To evaluate the protective effect of the SPL vaccine against *S. pneumoniae*, immunized mice and the unvaccinated groups were challenged with the wild-type bacteria via the IN route. Survival was monitored for 21 days after the challenge. After 4 and 8 days, mice in the unvaccinated groups died, while immunized mice remained alive for 21 days after the challenge.

### Antibody levels

The serum levels of immunoglobulin (Ig)G, IgM, and IgA in mice immunized with the SPL vaccine were evaluated by ELISA. Compared with the unvaccinated group, the immunized mice exhibited elevated levels of all three immunoglobulins. Notably, IgG levels were markedly higher than those of IgM and IgA. The differences observed were statistically significant in all mice ($P<0.0001$).

Immunized mice (SPL vaccine group) exhibited increased levels of IgM, IgG, and IgA compared to the unvaccinated group. Comparison of antibody levels revealed that IgG levels were higher than those of IgM and IgA. Data were presented as Mean±SE (Figure 2).

## Discussion

This study introduced and validated a novel vaccine development platform utilizing low-energy laser irradiation at 915 nm to inactivate *S. pneumoniae*. Our findings demonstrate that this method successfully achieves complete bacterial inactivation while meticulously preserving critical immunogenic epitopes, resulting in a whole-cell vaccine candidate that elicits a robust humoral immune response and confers complete protection in a murine challenge model. The superior performance of our laser-inactivated (SPL) vaccine, which achieved 100% survival and outperformed a commercial conjugate vaccine, underscores the significant potential of this technology.

The significant elevation in IgG levels observed in immunized mice is a particularly encouraging finding. Antibodies are pivotal for long-term immunity and effective opsonophagocytic clearance of *S. pneumoniae* [16, 17]. The shift from IgM to a strong IgG response indicates the activation of T-helper cell-dependent immunity and the development of immunological memory and high affinity antibodies [18, 19]. While our study focused on humoral immunity, the robust IgG response often implies concomitant T-cell help, suggesting that laser-inactivated whole-cell vaccines may potentially engage cellular immune mechanisms, an advantage over pure polysaccharide-based vaccines. Our findings are consistent with the study by Campo et al. who reported a pronounced increase in IgG responses following use of a whole-cell

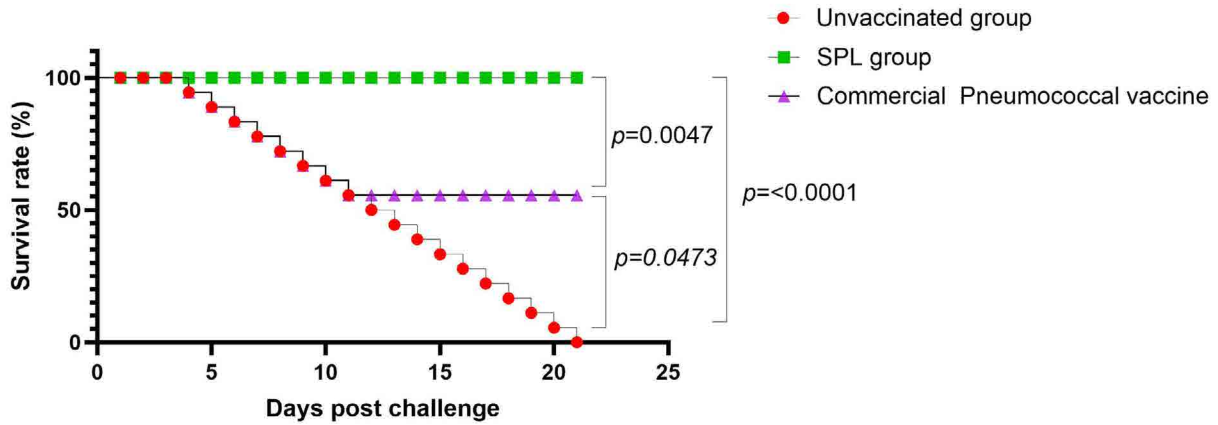


Figure 1. Log-rank (mantel-cox) test survival curves of mice post-challenge

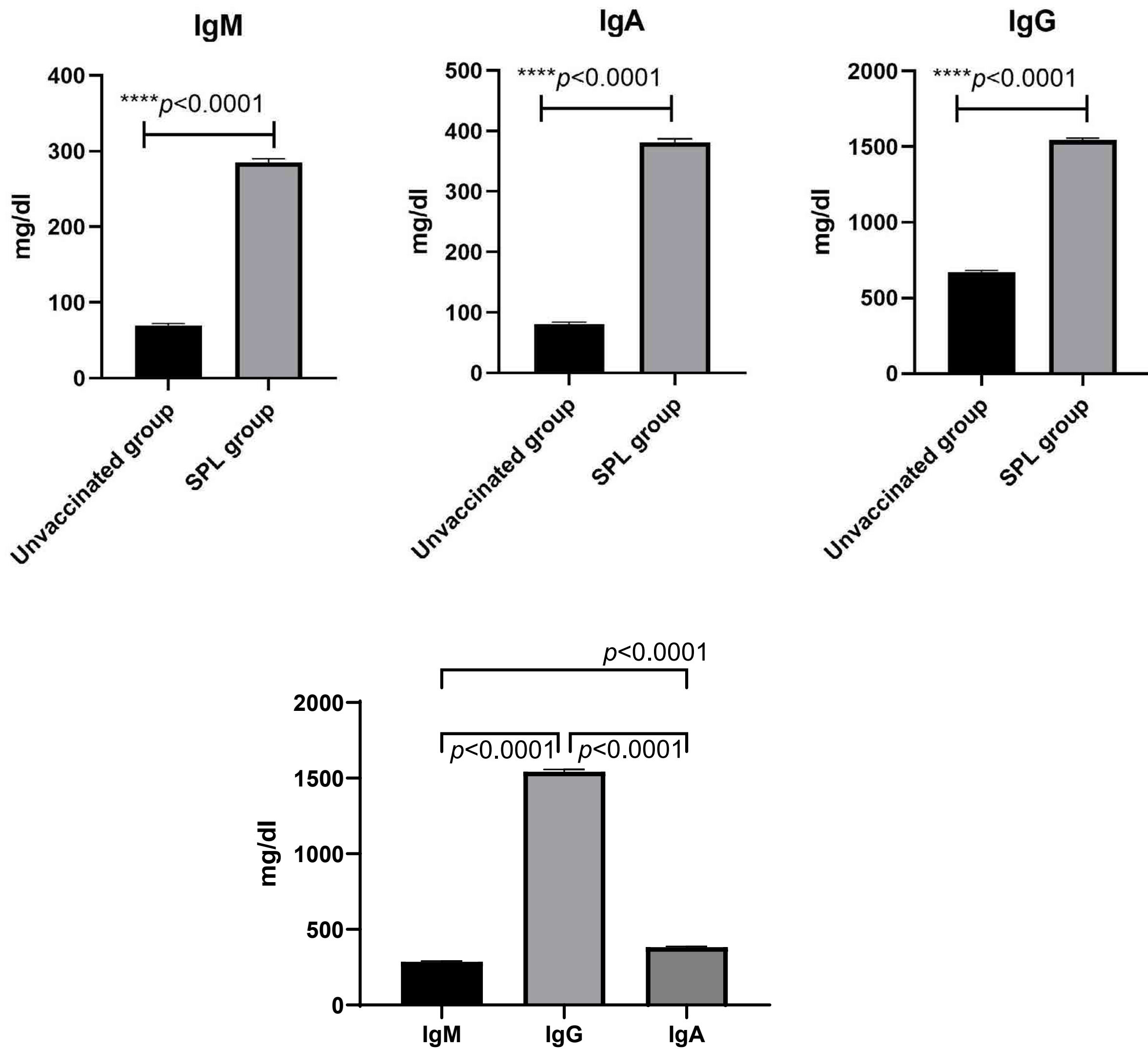


Figure 2. Evaluation of antibody levels after immunization

pneumococcal vaccine [20]. Our results align with the renewed interest in irradiated whole-cell vaccines, which are known to preserve a broader spectrum of antigens compared to heat or chemical inactivation. Gates et al. (2025) recently demonstrated that gamma-irradiated pneumococcal vaccines elicit superior immunogenicity compared to their heat- or chemically-inactivated counterparts, primarily due to better preservation of protein structures and conformational epitopes [9]. Similarly, Ko et al. (2021) showed that gamma-irradiated *S. pneumoniae* whole-cell vaccines induced protective immunity against pneumococcal colonization [10]. Our work builds upon this foundation by establishing laser irradiation as a viable, and potentially more accessible and controllable, alternative to gamma irradiation. The precise photophysical and photochemical interactions of laser light with bacterial cellular components (e.g. proteins, nucleic acids, and the cell wall) likely induce irreparable damage to genetic material and vital enzymes, leading to irreversible inactivation without causing the extensive protein denaturation associated with heat or formalin [21]. This "gentle" inactivation appears crucial for maintaining the immunogenic integrity of the bacterium.

The most striking outcome of our study was the complete protection (100% survival) afforded by the SPL vaccine, which exceeded the protection provided by the commercial conjugate vaccine (Prevenar 13). This superior efficacy, albeit in a mouse model, can be attributed to several factors. Whole-cell vaccines offer a vast and diverse antigenic repertoire, including conserved antigens common to different serotypes [22]. This broad antigenic protein can elicit immunity that is less susceptible to serotype replacement and could potentially provide cross-protection against a wider range of pneumococcal strains [20, 23].

The implications of developing a low-cost, serotype-independent pneumococcal vaccine are profound, particularly for low- and middle-income countries that bear the highest burden of pneumococcal disease [24]. Current conjugate vaccines (PCV10 or PCV13), while effective, are complex to manufacture and remain costly, limiting their universal accessibility and low cross-activity between serotypes [2]. A laser-inactivated whole-cell vaccine platform represents a paradigm shift towards a more sustainable and equitable solution for global pneumococcal disease prevention. The laser equipment used is relatively affordable and becoming increasingly portable, suggesting potential for decentralized manufacturing models in the future.

Despite these promising results, our study has limitations that warrant further investigation. Firstly, the protective immunity was evaluated in a murine model using a subcutaneous route; future studies should include models of pneumococcal colonization (nasopharyngeal) and pneumonia to fully assess the vaccine's efficacy across different disease manifestations. Secondly, while we documented a strong antibody response, the cellular immune component, particularly the role of Th1, Th17, and CD8+T cells, remains uncharacterized. Whole-cell vaccines are potent inducers of T-cell immunity [17, 25]. Therefore, investigating this axis is critical. Thirdly, the long-term durability of the immune response and the need for booster doses were not assessed. Finally, a more detailed analysis of the specific protein antigens recognized by immune serum (e.g. via immunoproteomics) would provide invaluable insights into the key protective antigens conserved by the laser inactivation process.

## Conclusion

In conclusion, we have established laser irradiation, specifically at a wavelength of 915 nm, as a highly effective and novel method for producing a protective inactivated whole-cell vaccine against *S. pneumoniae*. This SPL vaccine candidate elicited a protective immune response compared to the licensed vaccine. This difference is likely attributable to variations in serotypes rather than to insufficient efficacy of the commercial vaccine. By offering a potential solution to the cost and serotype-coverage limitations of current vaccines, this laser-based platform opens a promising new avenue for the development of accessible, broad-spectrum pneumococcal vaccines. Future work should focus on elucidating the precise mechanisms of immunity, optimizing the irradiation parameters, and validating these findings in higher animal models.

## Ethical Considerations

### Compliance with ethical guidelines

This study was approved by the Research Ethics Committees of University of Isfahan, Isfahan, Iran (Code: IR.UI.REC.1403.182).

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## Authors contribution's

All authors contributed equally to the conception and design of the study, data collection and analysis, interpretation of the results, and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

## Conflict of interest

The authors declared no conflict of interest.

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