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Investigating the Behavior of UVC Rays on the Inactivation of *Staphylococcus Aureus* from Simulated Contaminated Air Using a Probit Model

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Thesis

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Abstract

Today, one of the most significant health challenges is controlling airborne microbial agents. Utilizing ultraviolet (UV) rays is a method to eliminate these microbial agents and can be an effective approach for disinfecting hospitals. The main mechanism in which UV radiation works is not by directly eliminating bioaerosols, rather by preventing their proliferation through damaging the outer structure of their cells. As a result, bioaerosols cease to proliferate and eventually die due to cell death. In this study, our objective is to determine the optimal duration of UV irradiation and temperature and pH required to achieve efficient removal of S. aureus bacteria. A suspension of bacteria was prepared using the 0.5 McFarland standard. The desired contaminated air was produced and blown into the reactor using an air pump. Sampling was done using a membrane biosterile filter with a pore diameter of 0.22 micrometers in various UV irradiation time, temperature, and pH. The number of colonies grown from the samples was counted. The removal efficiency of S. aureus bacteria was >70% after 10 minutes of irradiation, >80% after 20 minutes, and >95% after 30 minutes at a constant rate. Also, the actual bacterial removal efficiency differs slightly from the removal efficiency predicted by the probit model.

Keywords: UVC; Staphylococcus Aureus; Remove Bacteria; Polluted Air

Abbreviation: UV Rays: Ultra Violet Rays.

Introduction

Today, one of the most important health challenges is preventing the spread of hospital infections. This is achieved by controlling airborne microbial agents that can be transmitted through droplets spread by exhalation. These droplets remain suspended in the air for some time [1-3]. These droplets may even enter the bloodstream through the lower respiratory system [4-6]. In addition, bacteria can produce toxins that initiate harmful processes in the host organism. These toxins inhibit protein synthesis, stimulate immune responses, and damage cell membranes. Bacterial cell wall compounds such as endotoxins and peptidoglycans have pro-inflammatory properties that can lead to respiratory symptoms like asthma, bronchitis and byssinosis. While most bacteria are usually harmless or even beneficial to us and the environment, problems arise when the concentration of potentially pathogenic bacteria exceeds the infectious dose. This infectious dose can vary significantly among different pathogenic species [7]. Staphylococcus bacteria are one of the main causes of nosocomial infections, accounting for 55-75% of these cases [8]. Due to concerns surrounding

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these infections, more focus has been placed on the use of ultraviolet radiation as a quick method to kill microbial agents. UV germicidal irradiation is a disinfection technique that uses UV radiation with short wavelengths of 100 to 280 nm [1,9,10]. The main mechanism by which UV radiation kills microorganisms is through the disruption of molecular bonds in DNA and RNA. This happens when photons are absorbed, leading to the formation of pyrimidine dimers from thymine and cytosine bases. It is important to note that UV radiation does not directly destroy bioaerosols, but prevents their proliferation by damaging the outer cell structure. As a result, bioaerosols can no longer reproduce and are not able to cause disease. Eventually they die due to cell death. Studies have shown that ultraviolet radiation emitted at a wavelength of 254 nanometers (nm) is particularly effective for this inactivation [11,12]. In addition, doses of 1.5-1.8 x 10^8 bacteria create the highest efficiency in exposure to UV with a wavelength of 254 nm for inactivating bacteria [13]. Increasing air humidity by trapping bacteria in water droplets increases the time bacteria stay suspended in the air. This will increase the efficiency of removing bacteria through UV irradiation [14]. Therefore, the contact time of airborne bacteria with ultraviolet rays is also an important factor [15]. when exposed to ultraviolet rays, based on similar studies [13]. UV radiation also provides rapid and significant inactivation by directly affecting the genetic material of microorganisms without leaving any by-products. This targeted mechanism makes UV radiation an effective and efficient method for microbial inactivation at a lower cost and without producing harmful byproducts [1]. Thus, this study aimed to determine the optimal humidity, temperature and exposure time required to achieve effective elimination of S. aureus bacteria using the UVC irradiation reactor.

Materials and Methods

UVC Device Specification

In the current study, an airtight reactor made of PVC with a polyamide coating was constructed. The reactor was equipped with a 9-watt UVC lamp with a wavelength of 254 nm. The reactor had dimensions of 14 cm in height, 13 cm in diameter, and a volume of 0.0018 cubic meters (Figure 1).

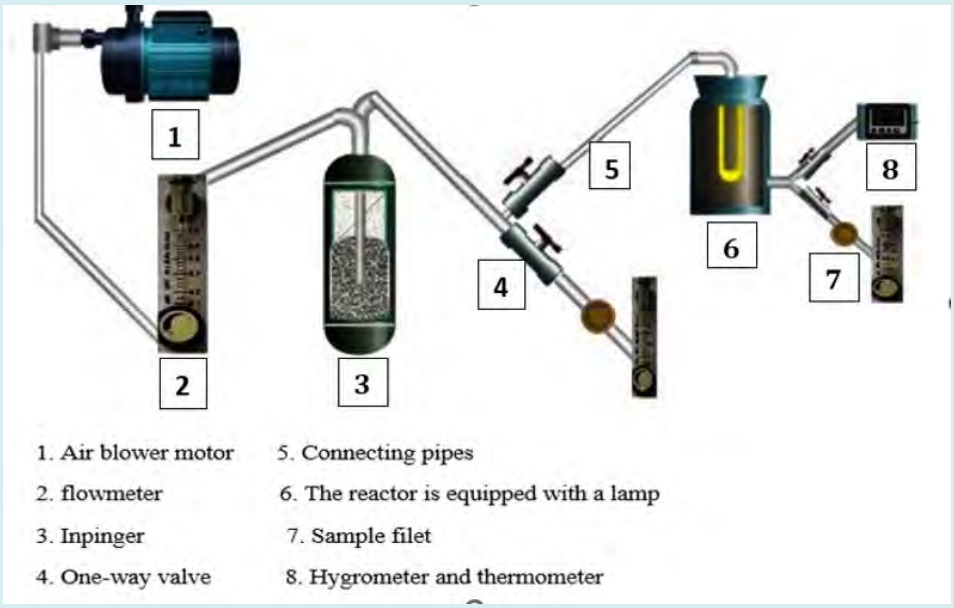

Figure 1: Schematic of UVC reactor for the removal of bacteria from the air stream.

Quantification of UVC Dose

To begin, a bacterial suspension of S. aureus was prepared from a S. aureus bank using a 0.5 McFarland standard [16]. The concentration of the suspension was then adjusted to 0.6 density at 620 nm wavelength using a spectrophotometer.

Preparation and Counting of Microorganisms

Air sampling was performed at the beginning and end of the reactor using a 47 mm CA Membrane bio sterile filter made of cellulose acetate with a 0.22-micron pore diameter. The filters were then placed on mannitol salt agar plates

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and incubated for 24-48 hours. After incubation, the grown colonies on the plates were counted using a colony counter device.

Bioaerosol Production and Air Sampling

The air was contaminated with the bacterial suspension using an air pump and impinger before entering the reactor at the desired flow rates. Air samples were then taken at a constant flow rate of 500 ml/s from the beginning and end of the reactor.

Number of Samples

Based on the capacity of the reactor and available laboratory facilities, a total of 78 samples were collected from the inlet and outlet air of the reactor over 11 experimental runs at different time intervals.

Statistical Analyses

In the present study, the distribution of bacteria in the air exposed to ultraviolet rays was analyzed by probit regression test.

Scenario Based Modelling

The probit regression model yielded an R2 value of 0.5818. Additionally, Eq1 was derived to model the efficiency of bacterial removal:

$$y = 0.3917x + 53.691 \quad (1)$$

Results and Discussion

The Effect of Sampling Time on the Performance of UVC Reactor

In order to determine the appropriate sampling time, air contaminated with S. aureus bacteria was sampled from the UVC reactor at time intervals of 0, 5, 10, 15, and 20 minutes, with a flow rate of 30 liters per minute. During all stages of the experiment, samples were taken using a cellulose acetate filter to collect bacteria from the air before and after exposure to ultraviolet rays. These samples were then analyzed by placing the filters containing the trapped bacteria on mannitol salt agar culture medium. The bacterial density was counted using a colony counter. The obtained results are shown in Figure 2.

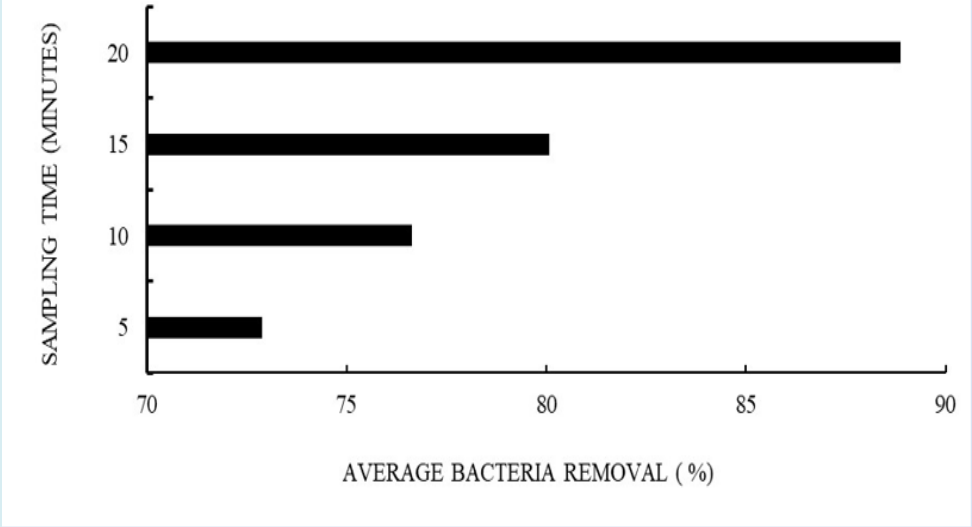

Figure 2: Removal efficiency of bacteria in the UVC reactor during different sampling periods.

As shown in Figure 2, the removal efficiency of S. aureus bacteria in the UVC reactor was 72.88±0.11% at 0-5 minutes; 76.64±4.17% at 0-10 minutes; 80.07±3.84% at 0-15 minutes and 87.87±2.40% at 0-20 minutes. This demonstrates that bacteria removal efficiency increased with longer sampling times.

The Effect of Elapsed Time on Bacteria Removal Since UVC Reactor Start

In order to determine the effect of UVC radiation, at air flow rates in the reactor of 25, 30, 35, and 40 liters per minute, the retention time inside the reactor was calculated

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to be 4.32, 3.6, 3.08, and 2.7 seconds, respectively, based on the reactor dimensions. Sampling was done at different time periods of 5-10, 10-15, 20-25, 30-35, and 40-45 minutes for each of the mentioned flow rates (Figure 3).

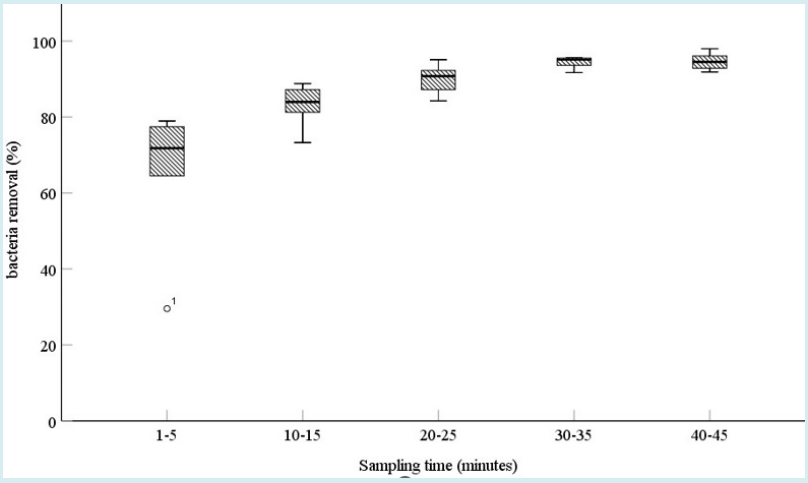

Figure 3: Percentage Removal of Bacteria at Sampling Time Intervals from the Reactor.

According to the results shown in the Figure 3, at 5-10 minutes the bacteria inactivation was 73.17 % on average; at 10-15 minutes it was 82.90%; at 20-25 minutes it was 91.36%. At 30-35 minutes it was recorded as 94.30%; and at 40-45 minutes it was 94.66. This demonstrates that the percentage of bacteria inactivation increased with longer UV radiation time at first. However, after 30 minutes of irradiation, the deactivation percentage took a constant trend. Consistent with the results of our study, Ewan Eadie et al.'s research showed that after 10 times applied at 5-minute intervals, for a total of 50 minutes, the efficiency of bacterial inactivation was 98.4% [17].

Determining Reactor Performance with 30-35 Minutes of UVC Radiation

In order to determine the effect of retention time on the removal of S. aureus bacteria from the air flow during 30-35 minutes after reactor start, contaminated air was fed into the reactor at inlet flow rates of 30, 40, 50, 60, and 70 liters per minute. Based on reactor dimensions, this resulted in retention times of 3.6, 2.7, 2.16, 1.8 and 1.5 seconds, respectively. The average bacterial removal vs exposure time are shown in Figure 4.

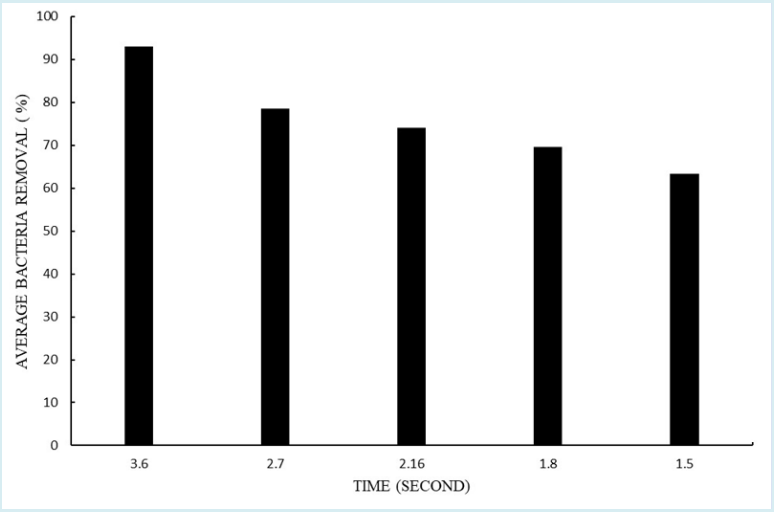

Figure 4: Average Removal of Bacteria at different Retention Times in the Reactor.

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According to Figure 4, the removal efficiency of S. aureus bacteria were: at 3.6 seconds retention time, 93.26%, at 2.7 seconds 78.65%; at 2.16 seconds 74.29%; at 1.8 seconds 69.71%; and at 1.5 seconds 63.59%. This demonstrates that bacteria removal increased with longer residence times in the reactor. In addition, the results obtained are consistent with Min Shang, et al. study's, which showed that the maximum inactivation of airborne viruses and bacteria was achieved with an optimal UVC radiation time of 30 minutes, aligning with the findings of our study [15].

Temperature and Humidity Changes

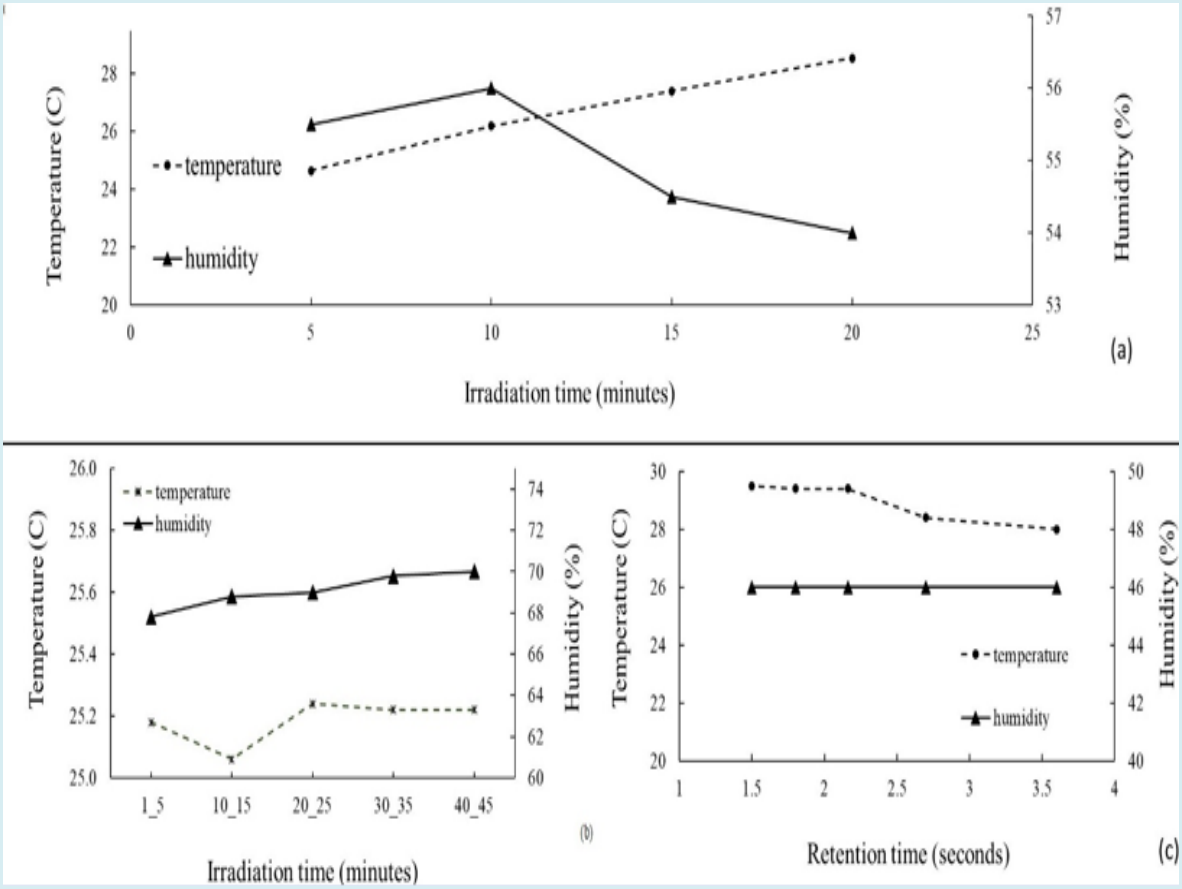

Figure 5: Shows the changes in temperature and humidity during the test stages.
Source: Temperature and Humidity Values at Sampling Times.

In Figure 5 (a), the average humidity was 55% ± 0.9%. In Figure 5 (b), the average increased to 69.07% ± 0.87% over time. However, in Figure 5 (c) the average humidity remained constant at 46%. The temperature data showed different trends between the sections. In Figure 5 (c), the average temperatures were 26.7°C ± 1.66 and 28.94°C ± 0.66; respectively, indicating an initial increase followed by a decrease. In contrast, Figure 5 (b) had an average of 25.3°C ± 0.07 that first decreased then increased before stabilizing. According to Aclaub, et al. in the removal of S. bacteria, both the high humidity and low temperature favors the removal efficiency [18].

Probit Regression Analysis

According to Probit Regression Analysis, the following results obtained, showing the actual bacteria amounts compared to the predicted model. As shown in Table 1, the actual efficiency of bacteria removal compared to the predicted efficiency from the Probit

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Probit	Number	Retention time	Irradiation time	Number of Subjects	Observed Responses	Expected Responses	Residual	Percentage removal efficiency observed	Percentage removal efficiency percentage
	1	5.4	1-5	176	124	43.402	80.598	29.55	75.34
	2	4.32	1-5	155	55	38.223	16.777	64.52	75.34
	3	3.6	1-5	375	79	92.476	-13.476	78.93	75.34
	4	3.08	1-5	71	16	17.509	-1.509	77.46	75.34
	5	2.7	1-5	234	66	57.705	8.295	71.79	75.34
	6	5.4	10-15	181	29	31.054	-2.054	83.98	82.84
	7	4.32	10-15	202	54	34.657	19.343	73.27	82.84
	8	3.6	10-15	1126	126	193.188	-67.188	88.81	82.84
	9	3.08	10-15	80	15	13.726	1.274	81.25	84.09
	10	2.7	10-15	336	43	57.648	-14.648	87.2	82.84
	11	5.4	20-25	211	27	23.843	3.157	87.2	88.7
	12	4.32	20-25	323	25	36.498	-11.498	92.26	88.7
	13	3.6	20-25	1189	109	134.355	-25.355	90.83	88.7
	14	3.08	20-25	87	5	9.831	-4.831	94.25	88.7
	15	2.7	20-25	339	26	38.306	-12.306	92.33	88.7
	16	5.4	30-35	266	13	18.702	-5.702	95.11	92.97
	17	4.32	30-35	181	15	12.726	2.274	91.71	92.97
	18	3.6	30-35	1232	55	86.62	-31.62	95.54	92.97
	19	3.08	30-35	89	4	6.257	-2.257	95.51	92.97
	20	2.7	30-35	345	22	24.256	-2.256	93.62	92.97
	21	5.4	40-45	256	14	10.562	3.438	94.53	95.87
	22	4.32	40-45	294	21	12.13	8.87	92.86	95.87
	23	3.6	40-45	1302	106	53.72	52.28	91.86	95.87
	24	3.08	40-45	98	2	4.043	-2.043	97.96	95.87
	25	2.7	40-45	357	14	14.73	-0.73	96.08	95.87

Table 1: Predicted and actual bacterial removal efficiency percentage.

Test differed by 5%< for irradiation times under 15 minutes. For irradiation times over 15 minutes, the difference between actual and predicted efficiency was also <5% (Figure 6). The figure below shows the process of removing bacteria.

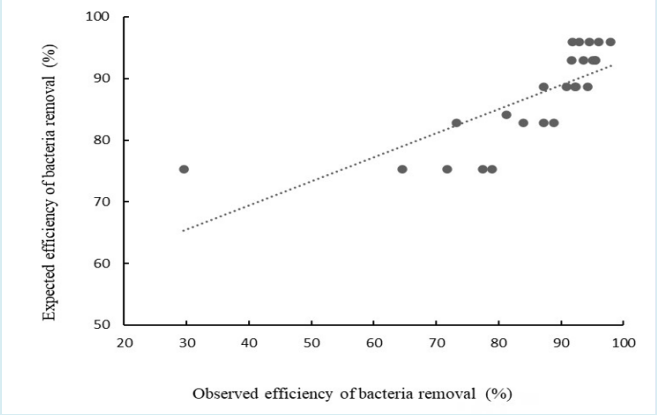

Figure 6: The model shows the actual amount of bacteria removed compared to the expected amount.

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Figure 6 shows the probit model comparing the actual amount of bacteria removed to the expected amount. This indicates that there is not much difference between the actual removal efficiency of 30% to 80% compared to the expected efficiency of 75% for removing Staphylococcus aureus bacteria from air, while the observed bacterial removal efficiency above 80% shows a 15% difference from the expected values. In general, a greater difference from the expected values was observed for the efficiency measurements above 80% compared to the measurements below 80%.

Conclusion

The results showed that the efficiency of removing bacteria depends on the exposure time to ultraviolet rays and the levels of humidity and temperature. Specifically, the bacteria removal efficiency first increases over time and then stabilizes. Additionally, higher humidity and lower temperature also improve bacteria removal by trapping bacteria in the air for longer periods, leading to increased removal efficiency.

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Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Contributions

All the authors contribute to the work. All authors read and approved the final manuscript.

Ethics Declarations

Ethics approval and consent to participate
Not applicable.

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

Authorship Statement

All authors meet the ICMJE authorship criteria. Sedighe Afrasiabi, ML and RR contributed to the study conception and design; SA acquired data; MK was a contributor in acquiring data; SA, ML, RR, and MK, conducted formal analysis, drafted, edited and reviewed the manuscript; All the authors have read and approved the final manuscript.

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