Saudi Journal of Biological Sciences 28 (2021) 1678–1686

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Efficacy of single versus multiple exposure by electromagnetic modalities on gram-negative and positive bacterial strains in an in-vitro model

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ARTICLE INFO

Article history:
Received 20 October 2020
Revised 29 November 2020
Accepted 3 December 2020
Available online 8 December 2020

Keywords:
Low level laser therapy
Magnetic therapy
Microwave diathermy
Gram negative bacteria
Gram positive bacteria
Photo-inhibitory
Electromagnetic spectrum

ABSTRACT

Objectives: The primary purpose of the recent experiment was to scrutinize the dissimilarity between single and multiple exposures by electrotherapeutic modalities to determine the development of Gram-positive and Gram negative bacteria spectrum.

Material and methods: Bacterial strains employed in this study were Gram-negative bacteria such as *Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella pneumonae* and Gram-positive bacteria such as *Staphylococcus aureus, Staphylococcus saprophyticus* and *Streptococcus pyogenes.* Fluence for Low level laser therapy (LLLT) (810 nm) was 40 J/cm^2 for 80 s, for microwave (MWD) a dosage of 100-Watt with duration of 5 min and for magnetic field therapy (MT) duration of 30 min with 100% intensity was used.

Results: Repeated Measures of analysis of variances (RANOVA) for within-subject effects was used to detect a global significant change within the means at dissimilar time points. The experiments of within-subjects revealed a significant difference within groups, df of (3, 40), F value of 39.38 and a p value less than 0.001, representing a significant variation between the three groups between pre and post exposures. There was a significant variation between single exposure and multiple exposures in the experimental sample's pre-post between the four groups with df (1, 40) f value of 2943.69 and p value less than 0.001. Scanning and Transmission electron microscopy images were also taken into account to determine the extent of damage caused to the bacterial cells surface topography in Gram negative and Gram positive spectrums.

Conclusion: The study demonstrated that single high exposure with the LLLT appears to have the most emphatic effect followed by exposure by MWD and MT.

1. Introduction

Gram negative and Gram positive bacteria are frequent source of wound infections in Middle-East and other Asian countries (Vijayakumar et al., 2018). The Middle Eastern countries are in specific confronting with problems like that of immigrants and impoerished living situation that has been urged by the civil war crisis. This might be a postulated cause for the propagation of bacterial resistance in these areas (Dandachi et al., 2019).

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2020.12.004
1319-562X/

Surgical site infections (SSI) in some Asian countries is yet again the foremost public health issue and is the second most commonly reported nosocomial contaminations worldwide (3). They also incur increased economic burden and psychological stress among the affected patients and families (Negi et al., 2015). It is reported that SSI following gastrointestinal surgery affects 25%-40% of the patients (Collaborative, 2017).

In Asian countries *Staphylococcus aureus* (50.4%) is the usual bacterial organism followed by *Escherichia coli* (23.02%), *Pseudomonas aeruginosa* (7.9%) that is mainly found wound cultures of the affected population. The Methicillin-resistant *Staphylococcus aureus* (MRSA) prevalence in the hospitals of Saudi Arabia was found to be around 40% which commonly affects the prognosis of the afflicted population in the region (El Amin and Faidah, 2012). To add further the increased hospital stay due to prevalence of infections and pressure ulcers is another factor that may delay the recovery of the patients and increase the chances for mortality and morbidity (Al-Hashemi, 2019).

On the contrary there is a necessity to ascertain some conservative methods for effective healing. The therapeutic modalities used by the therapist are mainly lasers and electromagnetic radiations which are some of the modalities that augments tissue regenerations and early rehabilitation of the person (Pe, 1999). There is an key role for physical therapist in the wound care team for the healing of chronic and acute wounds by offering a conservative, cost effective and non-surgical mode of treatments (Zhou et al., 2015; Pe, 1999).

LLLT which is commonly known as Low Level Laser therapy is as a potent modality of treatment for controlling the growth of Gram positive and Gram negative bacteria (Barboza et al., 2015; Dixit et al., 2019; Nussbaum et al., 2002). As they are low-cost devices and are progressively being used in health care system. It is usually postulated that absorption of laser light through chromophores causes alterations in cell physiology eventually leading to cascade of events resulting in cell damage of the bacteria's (Barboza et al., 2015).

In particular there are researches which states that laser probes with 810 nm wavelength (Dixit et al., 2019; Yuan et al., 2018) usually have less or no effect on *Staphylococcus aureus* or Gram-positive strains. Some researchers also emphasizes on contrary findings that LLLT is more effective on Gram positive strains than on Gram negative strains (Yuan et al., 2018).

In addition to lasers, Microwave diathermy (MWD) is also a physical therapy modality which operates in electromagnetic spectrum (Dixit et al., 2019). MWD uses electromagnetic radio waves with frequencies of 915 and 2456 MHz. Microwaves are selectively absorbed in tissues and this property is perfectly suited to treat pathologic procedures that take place in the muscles and adjacent fat (Effects TP). However, there is dearth in literature regarding the role MWD and its effects on Gram positive and Gram negative strains which needs to be further discovered (Dixit et al., 2019; Kumaran and Watson, 2015).

Magneto-therapy at the same time, is a harmless method of management of diseases in physical medicine (Valentinuzzi, 2008). The ability of the applied magnetic field to heal wound is well documented (Belik et al., 2014). Some researchers have also documented that magnetic fields influence the microorganisms in the oral cavity and may have bactericidal effects (Brkovic et al., 2015). Some studies have also found its beneficial effects in wound healing of diabetic foot with development of healthy granulation tissues (Ferroni et al., 2017). Though its effects on Gram negative and Gram positive strains still needs to be revealed and premeditated for future applications.

In physical medicine practice, there is a need to study the differences between single and multiple exposure on the growth of wide array of bacteria's cells count. Moreover, also to equate the bactericidal effect of laser, microwave diathermy and magneto-therapy on Gram positive and Gram negative bacteria spectrum and institute its usefulness as a worthwhile conservative and effective method of treatment.

2. Methods and material

2.1. Laser equipment

The experiment was conducted by means of a LLLT, category 4 Laser M 1000 plus (Level-Laser Co., Moglano Veneto-Milano, Italy). The equipment is usually furnished with an inflatable bottom foot switch, which is used to schedule the laser beam as per the requirement (Dixit et al., 2019). The LLLT parameters which were taken into account were as follows: The LLLT equipment was a company made standard semiconductor gallium –aluminium-arsenide (Ga-Al-As), producing a determined power of 1-Watt, constant wave (CW) at a wavelength of 810 nm and a frequency of 500 Hz. The laser beam had a spot size of 0.5 cm^2 with a duty cycle of 50% and voltage of 240 V (Dixit et al., 2019). The optimum fluence decided for the LLLT for each point to expose the wide spectrum of Gram-positive and Gram-negative bacterial strains was 40 J/cm^2 for 80 s(s) with non-contact method. The LLLT instrument created a beep sound when the management period finished. The therapist was robed in specific goggles as a shield from the laser's stream of light. As per the study protocol the accuracy of the LLLT beam production was tested before the commencement of the study by means of a specified photodiode paraphernalia called as dosimeter. The intrinsic dosimeter was utilized to homogenize the probe beforehand and during the experiment.

2.2. Microwave diathermy equipment

The microwave diathermy (MWD) unit used was Radarmed 950 + device (Enraf nonius, Rotterdam, The Netherlands) to perform the experiment (Dixit et al., 2019). It can generate with a constant method a power up to 250 Watt incorporating a pulsed discharge technique up to an all high of 1500 Watt (W). The machine was furnished with easy swapping between constant and pulsed modes during the experiment. The desired inbound factors remains noticeable during the process of the experiment with an intrinsic LCD system of the machine. During the experiment a constant discharge method with the frequency of 2450 MHz ± 50 MHz incorporating a power of 100 W for 5 min (min) by means of non-contact method was implemented. The variation of temperature in the suspensions were observed with a laser thermometer.

2.3. Magnetotherapy equipment

The experiment was conducted with Level health waves is an innovative magnetotherapy equipment (Level-Laser Co., Moglano Veneto-Milano, Italy). Level health waves contains 60 pre-installed and completely user editable programs and other 39 programs which can be saved by the user. The level health wave's generator consists of two independent outputs in the emission and asynchronous operating parameters and a magnetic field intensity from 10 to 100 Gauss. Magnetotherapy system is composed of generator and 2 cylinders with a diameter 65 cm with wooden bed with sliding guides. For the experiment, the channel 1 and 2 the intensity was set to 100% for 30 min with frequency of 120 Hz using non-contact method.

2.4. Bacterial samples

This study was conducted on three commonly found organisms in Gram-negative bacterial strains such as *Klebsiella pneumonae, Escherichia coli* and *Pseudomonas aeruginosa* and on three Gram-positive bacterial strains such as *Staphylococcus aureus, Staphylococcus saprophyticus, and Streptococcus pyogenes*. The basic culture technique employed for all the strains was in Brain Heart Infusion (BHI) liquid broth with a temperature of 37 °C. The preparation of the bacterial samples were done in a suspension equivalent to 1.5×10^2 colony forming units (cfu)/mL in BHI broth for the treatment of laser, microwave and magnetotherapy.

2.5. Experimental protocol

200 µl (microliter) of above bacterial strains were poured into three different 96 wells plate for the irradiation with laser, microwave and magnetotherapy. A laser beam was exposed on the first 96 wells plate containing 200 µl, the samples were placed in an individual second 96 wells plate which were then exposed to microwave radiations. The samples for magnetotherapy exposure were on the third 96 wells plate. After irradiations on day 1, 100 µl of the bacterial suspension from each wells were cultured by pour plate procedure on BHI agar plate and incubated at 37 °C for 24 h. Bacterial colonies were counted and converted into CFU (Dixit et al., 2019). 100 µl BHI broth was added in each well to make it 200 µl. The above-mentioned samples, which were irradiated with laser, microwave and magnetotherapy for three continuous days, were also analyzed as described above.

2.6. Transmission electron microscopy (TEM)

Based on electrotherapeutic efficiency, untreated bacterial strains and bacterial strains treated for 4 groups were subjected to transmission electron microscopy to observe their activity on the morphology of the bacterial cell as described previously (Hartmann et al., 2010). Test organisms *S. aureus* and *E. coli* were grown in 2 ml of BHI broth in different tubes for 18 h at 37 °C. Broth was further centrifuged at 5000xg and pellets were washed two times with PBS, fixed with glutaraldehyde and resuspended in 500 µl PBS. Formvar coated 300-mesh copper grids were coated with 5 µl sample suspension for 10 min with sterile tweezers and rinsed once with sterile Milli-Q water to eradicate imprudent non-coated material. Negative staining was accomplished by immersing coated grids for 20 s in 1% uranyl acetate followed by destaining in sterile Milli-Q water and air drying. Grids were detected directly with a JOEL CO-Japan's JEM 100 transmission electron microscope operating at 80 kV.

2.7. Scanning electron microscopy (SEM)

The SEM has a large depth of fields and allows large number of samples to be focused in one time and produces an image that is a good representative of three-dimensional samples (Eid et al., 2019). A pathologist independently without the prior knowledge of the strains and level of exposure given to the samples examined the specimen of the four groups.

Firstly, SEM samples preparation was done in centrifuge tubes. Then the Specimens of bacteria were immediately fixed in 2.5% (wt/vol) phosphate-buffered glutaraldehyde, pH 7.4 at 4 °C for 4 h. Samples were also post-fixed in 1% phosphate-buffered osmium tetroxide, pH 7.4 for 1 h. After washing and dehydration in ascending grades of ethanol, critical-point drying was accomplished using the EMITECH-K850 critical-point dryer. The samples were mounted on aluminum mounts with silver glue and then sputtered with gold coated by BOC EDWARDS SCANCOAT. The specimens were examined by a Jeol scanning electron microscope JSM-6390LV, Japan (Eid et al., 2019).

2.8. Data-analysis

Log transformation was applied to the skewed data. To maintain the uniformity in the data the log units were converted by elevating 10 to the power of the number. The statistics in the study were presented as mean and standard deviation which are considered as a measure of central tendency and dispersion. The exploration was done using Statistical Package for the Social Sciences (SPSS) 20. The repeated-measures of analysis of variance (RANOVA) was employed to assess the changes in the outcome measures for various dosages for the test and the control group with respect to single and multiple irradiations of the samples between the four groups (i.e Laser, MWD, MT and control). The statistically significance was considered at a probability value of p less than 0.05. Moreover, as the Mauchly's test of sphericity was significant (p less than 0.05), which states that the sphericity assumption was violated, hence the Greenhouse–Geisser correction factor (GG) was considered to interpret the results. In the RANOVA tests the degrees of freedom were reported as (degree of freedom (df) 1, degree of freedom (df) 2 and F values with respective p values. The degree of the difference in outcomes between the 4 groups was compared by the effect sizes under the treatment groups and computed as with the description given by Cohen et al. (Coe, 2002). The relative and absolute changes in the samples were also analysed.

2.9. Ethical approval

The study was approved by the university Ethical Committee of the Scientific Research with the approval number (ECM#2019–10 7)—(HAPO-06-B-001).

3. Results

The evaluation of single versus multiple dosages of laser, MWD, MT (treatment group) and control group is presented as mean and standard deviation of Gram negative and Gram positive strains cells count in table 1. The effect of laser, MWD, MT and control (no treatment) on growth of each individual bacteria in Gram negative and Gram positive spectrum is depicted as mean and standard deviation under Table 2.

The results of the study were interpreted using RANOVA for within-subject effects measuring a global noteworthy dissimilarity amid the means at dissimilar periods. The RANOVA measure of within-subjects effects revealed a significant difference within groups, df of [3,40], F value of 39.38 and a p value less than 0.001, specifying a significant difference between the three groups between Pre and post exposures. There was a significant difference between single exposure and multiple exposures in the experimental sample's pre-post between the four groups with df [1,40] f value of 2943.69 and p value less than 0.001. There was a significant interaction between the group and exposures before and after the experiment with df [3,40], f value of 8.63 and p value less than 0.001.

The images taken by the scanning electron microscope (SEM) were also taken into account to determine the extent of damage caused to the bacterial cells by surface topography in Gram negative and Gram positive spectrums (Fig. 3). The structure morphology of *S. aureus* and *E. coli* was examined under the scanning microscope to observe the changes after the single and multiple treatments shown in Fig. 3. Untreated (control) cells showed clear morphology (Fig. 3. A & 3D), which was not seen in case of samples

Table 1
Comparison of single versus multiple dosages of treated group and control group on mean and standard deviation of Gram negative and Gram positive strains cells count.

Group	SINGLE DOSE (Cfu/mL)		MULTIPLE DOSE (Cfu/mL)	
Control	*Pre (Mean ± SD) CI*	*Post (Mean ± SD) CI*	*Pre (Mean ± SD) CI*	*Post (Mean ± SD) CI*
Gram Negative organisms	162.18 ± 1.03 [161.36–163.01]	295.12 ± 1.04 [294.29–295.95]	162.18 ± 1.03 [161.36–163.01]	1380.38 ± 1.02 [1379.56–1381.20]
Gram Positive organisms	165.96 ± 1.03 [165.14–166.78]	288.40 ± 1.07 [287.54–289.26]	165.96 ± 1.03 [165.14–166.78]	1445.44 ± 1.07 [1444.58–1446.30]
Group				
Laser	*Pre (Mean ± SD) CI*	*Post (Mean ± SD) CI*	*Pre (Mean ± SD) CI*	*Post (Mean ± SD) CI*
Gram Negative organisms	162.18 ± 1.03 [161.36–163.01]	162.18 ± 1.12 [161.28–163.08]	162.18 ± 1.03 [161.36–163.01]	1202.26 ± 1.07 [1201.40–1203.12]
Gram Positive organisms	165.96 ± 1.03 [165.14–166.78]	134.9 ± 1.07 [134.04–135.76]	165.96 ± 1.03 [165.14–166.78]	1071.52 ± 1.12 [1070.62–1072.41]
Group				
MWD	*Pre (Mean ± SD) CI*	*Post (Mean ± SD) CI*	*Pre (Mean ± SD) CI*	*Post (Mean ± SD) CI*
Gram Negative organisms	162.18 ± 1.03 [161.36–163.01]	169.82 ± 1.12 [168.92–170.72]	162.18 ± 1.03 [161.36–163.01]	1230.27 ± 1.07 [1229.41–1231.13]
Gram Positive organisms	165.96 ± 1.03 [165.14–166.78]	172.78 ± 1.17 [171.84–173.72]	165.96 ± 1.03 [165.14–166.78]	1174.90 ± 1.12 [1174.01–1175.80]
Group				
MT	*Pre (Mean ± SD) CI*	*Post (Mean ± SD) CI*	*Pre (Mean ± SD) CI*	*Post (Mean ± SD) CI*
Gram Negative organisms	162.18 ± 1.03 [161.36–163.01]	165.96 ± 1.15 [165.04–166.88]	162.18 ± 1.03 [161.36–163.01]	1230.27 ± 1.04 [1229.44–1231.10]
Gram Positive organisms	165.96 ± 1.03 [165.14–166.78]	144.54 ± 1.08 [143.68, 145.40]	165.96 ± 1.03 [165.14–166.78]	933.25 ± 1.20 [932.29–934.21]

*SD- standard deviation .CI- confidence interval at 95%, Cfu/mL—Colony-forming units per milliliter.

treated with single exposure (Fig. 3B & 3E) and multiple time exposure (Fig. 3C & 3F). Unlike control, the cells in treated samples were clearly showed the damage of the cell membrane was observed.

Transmission electron microscopy (TEM) showed characteristic morphological changes in both the strains *S. aureus* and *E. coli* after treatment. In contrast with untreated growth control (Fig. 1A. 1B & 2A, 2B), showing cell uniform cytoplasmic density (asterisks) and intact cell double membranes (black arrows). Bacterial cells were undergoing division. We observed that bacterial strains treated with magnetic therapy showing focal translucent (asterisks) of the cytoplasm and intact cell double membranes (black arrows). Bacterial cells were undergoing division (white arrow heads) (Fig. 1C. 1D & 2C, 2D). The bacterial strains treated with microwave exposure showing abnormal cytoplasm (asterisks) and detached and damaged from the cell membranes (black arrows). Shrunken cells were also seen and bacterial cells were undergoing division (white arrow heads) (Fig. 1E, 1F & 2E, 2F). Laser exposure showing misshapen cells with pleomorphic and damaged cytoplasm (asterisks). Detached, Shrunken and damaged from the cell membranes (black arrows) were also seen. Bacterial cells were undergoing division (white arrow heads) (Fig. 1G. 1H & 2G, 2H).

The test for effect size which usually defines the clinical significance was evaluated for laser, MWD and MT group for Gram negative and Gram positive bacteria with single and multiple dosages, it showed a large treatment effect of −0.99 respectively. The classification as given by Cohen et al. is as follows: less than0.2 = trivial effect; 0.2–0.5 = small effect; 0.5–0.8 = moderate effect; >0.8 = large effect was used (Coe, 2002). In the current study, negative effect size stipulated a decrease in the bacterial cell count and vice versa. Table 3 represents in depth analysis of bacterial samples (*E. coli* and *S. aureus*) by TEM post treatment by LLLT, MT and MWD. The relative and absolute differences between the groups are discussed under the Table 4.

4. Discussions

The primary reason to undertake the study was to explore the inhibitory efficiency of single and multiple exposure against the growth of wide array of bacteria with electrotherapeutic devices.

Electrotherapeutic modalities have acclaimed the role for enhancing the wound healing by shortening the duration for the healing process (Coe, 2002; Abbas et al., 2011). But there are only a small number of reports which have scrutinized the activity of these modalities on the micro-organisms growth with selective bacteria's (Dixit et al., 2019; Hunckler and de Mel, 2017). In the present study which comprised of Gram-positive strain and Gram-negative strain, post exposure the organisms responded differently to various electrotherapeutic modalities (Table 1-2,4). Usually Gram negative bacteria are related with significant morbidity and mortality particularly in patients with intensive care units (ICU) (Della-Latta et al., 2011). Hence the aforementioned therapy might be useful in the areas were clinicians witness a significant antimicrobial resistance.

The results in the present study revealed that LLLT is a very potent tool to have a broad-spectrum effect on both Gram positive and Gram negative strains. The high fluence of 40 J/cm^2 with the wavelength of 810 nm appears to be an effective prescription to decrease the growth of wide array of bacteria's (Table 1). The fluence of 40 J/cm^2 with single exposure of the micro-organisms appears to be more effective in triggering not only a decrease in the cell counts but also altering the cell structures of the microorganisms (Figs. 1-3). On the contrary multiple exposure with LLLT appears to be less effective than single dose irradiation. Results for Gram positive strains are in accordance with other researches (Dixit et al., 2019; Yuan et al., 2018; Percival et al., 2015) which are assumed to be more susceptible to cell damage than Gram negative strains. The LLLT irradiation can make the physiological function of cells vulnerable to extensive loss of metabolic activity of cell and finally to physical breakdown (Yuan et al., 2018).

Laser with single dose exposure showed no relative increase or decrease (0%) under Gram negative strain versus increase of 82% in control group and a decrease of 19% under Gram positive group MWD versus increase of 74% in control group (Table 4).

Another electrotherapeutic modality, that is, MWD is quiet less explored in the arena of wound healing. Radiations from the MWD is released as a stream from a protuberance which is easily absorbed by the tissues which are rich in water more effectively

Table 2
Mean cells count, standard deviation and confidence intervals of the Gram negative and Gram positive microorganisms in control and treated groups.

Group	(cell counts in Cfu/mL)	(cell counts in Cfu/mL)
Control	*Pre (Mean ± SD)CI*	*Post (Mean ± SD)CI*
Gram Negative organisms		
Pseudomonas aeruginosa	154.88 ± 1[154.08–155.68]	630.96 ± 3.02[628.54–633.38]
Escherichia coli	165.96 ± 1[165.16–166.76]	660.69 + 2.95[658.33–663.05]
Klebsiella pneumonae	162.18 ± 1[161.38–162.98]	645.65 ± 2.95[643.29–648.01]
Gram Positive organisms		
Streptococcus pyogenes	158.49 ± 1[157.69–159.29]	602.56 ± 3.16[600.03–605.09]
Staphylococcus aureus	165.96 ± 1[165.16–166.76]	676.08 ± 3.02[673.66–678.50]
Staphylococcus saprophyticus	165.96 ± 1[165.16–166.76]	660.69 ± 3.31[658.04–663.34]
Group		
Laser	*Pre (Mean ± SD) CI*	*Post (Mean ± SD) CI*
Gram Negative organisms		
Pseudomonas aeruginosa	154.88 ± 1[154.08–155.68]	457.09 ± 4.07[453.83–460.35]
Escherichia coli	165.96 ± 1[165.16–166.76]	426.58 ± 4.79[422.75–430.41]
Klebsiella pneumonae	162.18 ± 1[161.38–162.98]	436.52 ± 3.72[433.54–439.50]
Gram Positive organisms		
Streptococcus pyogenes	158.49 ± 1[157.69–159.29]	354.81 ± 4.27[368.12–374.96]
Staphylococcus aureus	165.96 ± 1[165.16–166.76]	436.51 ± 3.72[433.53–439.49]
Staphylococcus saprophyticus	165.96 ± 1[165.16–166.76]	371.54 ± 4.27[368.12–374.96]
Group		
MWD	*Pre (Mean ± SD) CI*	*Post (Mean ± SD) CI*
Gram Negative organisms		
Pseudomonas aeruginosa	154.88 ± 1[154.08–155.68]	467.74 ± 3.63[464.84–470.65]
Escherichia coli	165.96 ± 1[165.16–166.76]	446.68 ± 4.68[442.94–450.43]
Klebsiella pneumonae	162.18 ± 1[161.38–162.98]	467.74 ± 3.80[464.70–470.78]
Gram Positive organisms		
Streptococcus pyogenes	158.49 ± 1[157.69–59.29]	398.11 ± 4.17[394.77–401.45]
Staphylococcus aureus	165.96 ± 1[165.16–166.76]	446.68 ± 3.55 [443.84–449.52]
Staphylococcus saprophyticus	165.96 ± 1[165.16–166.76]	512.86 ± 3.89[509.75–515.97]
Group		
MT	*Pre (Mean ± SD) CI*	*Post (Mean ± SD) CI*
Gram Negative organisms		
Pseudomonas aeruginosa	154.88 ± 1[154.08–155.68]	467.74 ± 3.89[464.63–470.85]
Escherichia coli	165.96 ± 1[165.16–166.76]	426.58 ± 4.90[422.66–430.50]
Klebsiella pneumonae	162.18 ± 1[161.38–162.98]	467.74 ± 3.80[464.70–470.78]
Gram Positive organisms		
Streptococcus pyogenes	158.49 ± 1[157.69, 159.29]	338.84 ± 3.24[336.25, 341.43]
Staphylococcus aureus	165.96 ± 1[165.16, 166.76]	389.05 ± 3.63[386.15–391.96]
Staphylococcus saprophyticus	165.96 ± 1[165.16, 166.76]	407.38 ± 4.17[404.04–410.72]

[#]SD- standard deviation. CI- Confidence Interval at 95%, Cfu/mL—Colony-forming units per milliliter.

than shortwave frequency (Waldman, 2009). In the present study MWD single exposure of 100 W for 5 min showed a relative increase of 4.71% under Gram negative strain versus increase of 82% in control group and an increase of 4.11% under Gram positive group MWD versus increase of 74% in control group (Table 4). In a study authors found MWD to have decrease in cell counts among Gram negative and Gram positive strains though the results remained insignificant (Dixit et al., 2019). The authors in the above-mentioned study used multiple exposure that might be a reason for their findings. Another study which used microwave radiations with power of 600 W showed reduction in cell count of *E.coli* but no significant differences in cell density of the organisms (Woo et al., 2000).

Magnetotherapy on the contrary has well established evidences that states MT can be an effective treatment to promote wound healing and musculoskeletal repairs (Brizhik et al., 2016). In the present study authors found that MT can be an effective tool against most common Gram-positive strains causing a relative reduction of 13% while the Gram-negative strains increased 2.33% only (Table 4).

Previously MT has been found to be effective against certain Gram positive and fungal strains isolated from the oral cavity (Brkovic et al., 2015). Another study investigated the influence of fixed or static magnetic fields on *E. coli* which was derived from the urine samples of the patients, didn't observe any significant difference between the control and treated samples. A plausible reason could be low intensity used in the experiment (Mousavian-Roshanzamir and Makhdoumi-Kakhki, 2017).

In the present study the authors found that single and multiple irradiations have using LLLT, MWD, MT not only have clinically significant effects but also had bactericidal effects (Table 2). Though it was relatively evident that single exposure was more effective as compared to multiple exposure in having antimicrobial effects. Moreover, the secondary objective was to compare the bactericidal effect of laser, microwave diathermy and magneto-therapy on Gram negative and Gram positive spectrum of bacteria and institute its usefulness as an alternate conservative and effective mode of treatment. The results of the present study deliniated that wavelength of 810 nm with the fluence of 40 J/cm^2 in LLLT, 100 W in MWD, and 100% intensity in MT were effective in reducing or controlling the increase in cell counts of Gram negative and Gram positive micro-organisms as compared to the control group. Though some studies have elucidated the bactericidal effects of LLLT (Mousavian-Roshanzamir and Makhdoumi-Kakhki, 2017; De et al., 2006) with specific wavelengths in limited ways (Nussbaum et al., 2003), still it was essential to undertake the study to explore the efficacy and properties of MWD (Dixit et al., 2019) and MT against wide array of bacteria's.

The determination of the molecular structures of the samples were the most striking finding in the study. The interactions of the bacterial samples with the physical therapy modalities and processes including structural and functional relationships at cellular level (Fajardo et al., 2016) were well defined using a SEM technique (Fig. 3) and were further augmented by the TEM technique (Figs. 1-2). Our understanding on the level of damage induced by the modalities in comparison to the control was further amplified (Table 3) and we can understand the level of damage induced by the respective dosages of the physical therapy modalities used. The images with TEM actually complemented the present research techniques with a three-dimensional view (Graham and Orenstein, 2007), and our ability to study different components of the bacterial cell damage that was high in LLLT as compared to MWD and MT.

The safety of these electrotherapeutic devices in a clinical scenario have already been established (Abbas et al., 2011; Graham

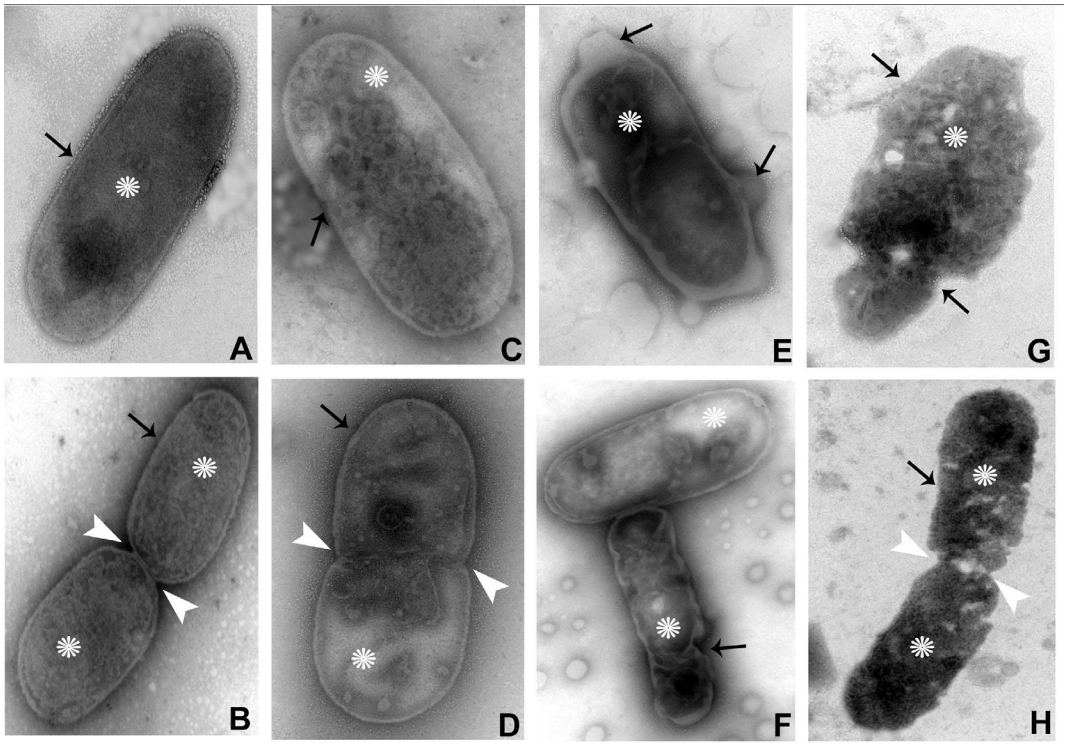

Fig.1. Effect on cellular morphology of *E. coli:* Changes in the cellular morphology was observed by Transmission Electron Microscope. **A & B**: untreated control showing cell uniform cytoplasmic density (asterisks) and intact cell double membranes (Black arrows). Bacterial cells were undergoing division (White arrow heads). **C & D**: treated cells after magnetic exposure showing focal translucent (asterisks) of the cytoplasm and intact cell double membranes (Black arrows). Bacterial cells were undergoing division (White arrow heads). **E & F**: treated cells after microwave exposure showing abnormal cytoplasm (asterisks) and detached and damaged from the cell membranes (Black arrows). Shrunken cells were also seen. Bacterial cells were undergoing division (White arrow heads). **G & H**: treated cells after laser exposure showing misshapen cells with pleomorphic and damaged cytoplasm (asterisks). Detached, Shrunken and damaged from the cell membranes (Black arrows) were also seen. Bacterial cells were undergoing division (White arrow heads).

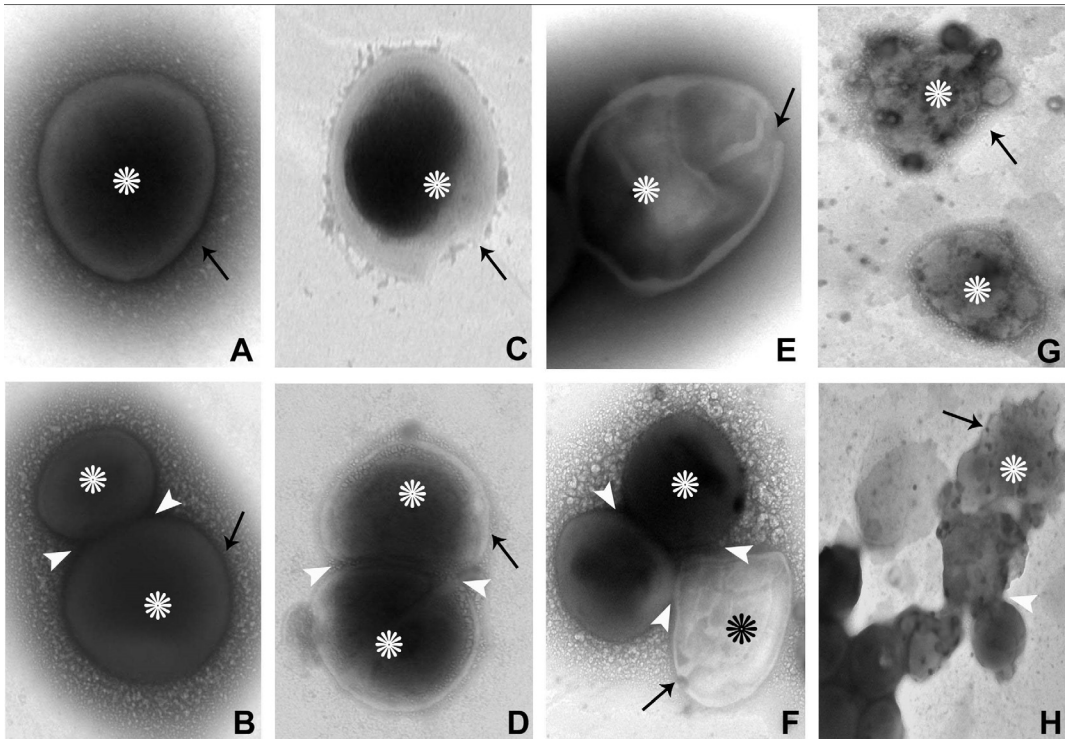

Fig. 2. Effect on cellular morphology of *S. aureus:* Changes in the cellular morphology was observed by Transmission Electron Microscope. **A & B**: untreated control showing cell uniform cytoplasmic density (asterisks) and intact cell double membranes (Black arrows). Bacterial cells were undergoing division (White arrow heads). **C & D**: treated cells after magnetic exposure showing focal translucent (asterisks) of the cytoplasm and intact cell double membranes (Black arrows). Bacterial cells were undergoing division (White arrow heads). **E & F**: treated cells after microwave exposure showing abnormal cytoplasm (asterisks) and detached and damaged from the cell membranes (Black arrows). Shrunken cells were also seen. Bacterial cells were undergoing division (White arrow heads). **G & H**: treated cells after laser exposure showing misshapen cells with pleomorphic and damaged cytoplasm (asterisks). Detached, Shrunken and damaged from the cell membranes (Black arrows) were also seen. Bacterial cells were undergoing division (White arrow heads).

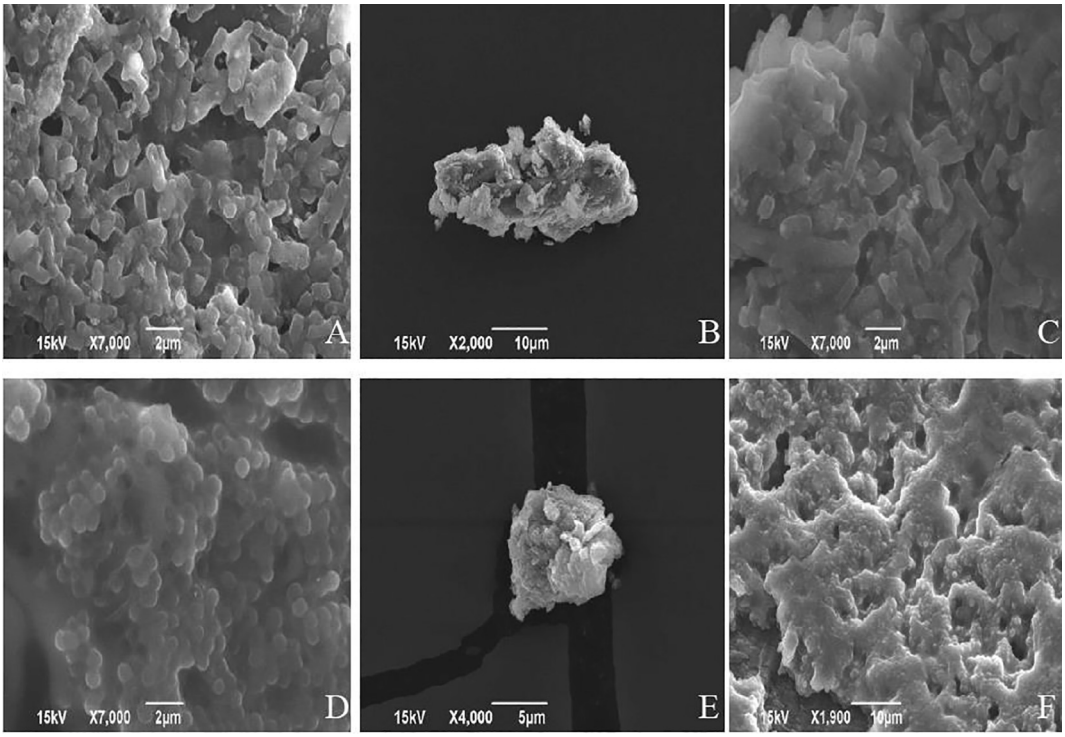

Fig. 3. Effect of laser therapy on cell morphology of *S. aureus* and *E. coli:* Changes in the cellular morphology was observed by Scanning Electron Microscope. A: *E. coli* control cell, B: single exposure showed the damage of the *E. coli* cell membrane, C: Multiple exposure showed damage of *E. coli* cell membrane. D: *S. aureus* control cell, E: single exposure showed the damage of the *S. aureus* cell membrane, F: Multiple exposure showed damage of *S. aureus* cell membrane.

Table 3
Analysis of the samples with TEM morphological features for magnetic, microwave, and laser exposure–treated bacteria.

Escherichia coli cells					
No	Aspects	Control	MT	MWD	LLLT
1	Shape of the cells	Oval roads	Oval roads	Oval roads	Oval roads
2	Cell membrane	intact cell double membranes	intact cell double membranes	detached and damaged from the cell membranes	Detached, Shrunken and damaged
3	Cytoplasm	uniform cytoplasmic density	focal translucent	abnormal cytoplasm	Abnormal, pleomorphic and damaged
4	Bacterial cell division	undergoing division	undergoing division	undergoing division	undergoing division
5	Percentage of the cell damaging	0%	20%	50%	70%
***Staphylococcus aureus* cells**					
No	**Aspects**	**Control**	**Magnetic**	**Microwave**	**Laser**
1	Shape of the cells	Spherical	Spherical	Spherical	Spherical
2	Cell membrane	intact cell double membranes	intact cell double membranes	detached and damaged from the cell membranes	Detached, Shrunken and damaged
3	Cytoplasm	uniform cytoplasmic density	focal translucent	abnormal cytoplasm	Abnormal, pleomorphic and damaged
4	Bacterial cell division	undergoing division	undergoing division	undergoing division	undergoing division
5	Percentage of the cell damaging	0%	15%	40%	60%

TEM- Transmission Electron Microscopy, MT- Magneto therapy, MWD- Microwave Diathermy, LLLT- Low Level Laser Therapy.

and Orenstein, 2007) but there was also a need to comprehend the mechanism examining in what manner the Gram positive and Gram negative bacterial spectrum responded to single and multiple irradiations. In the present study it was evidently noticed that LLLT with the aforementioned fluence was more effective with single exposure than multiple exposure to Gram positive and Gram negative strains. Similar findings were also noticed with MWD and MT. Though the study elucidated a specific mechanism which is unclear, hence still there is a need for further experiments to explore the mechanism responsible.

5. Conclusion

The experiment has demonstrated that single exposure with LLLT appears to have an emphatic effect on Gram-positive and Gram-negative bacteria. MT and MWD also had a clinically significant effect on the strains as compared to multiple exposure. The imperative findings in the study establishes the use of electrotherapy modalities as a non-invasive and alternate substitute for the population experiencing drug resistance or wound dehiscence.

Table 4
Mean absolute change and relative change (%) in control and treatments groups.

Group	Single Dose		Multiple Dose	
	Absolute change Cfu/mL	Relative change	Absolute change Cfu/mL	Relative change
Control				
Gram negative	132.94	81.97%	1218.20	751.14%
Gram positive	122.44	73.78%	1279.48	770.96%
Laser				
Gram negative	0	0	1040.08	641.31%
Gram positive	−31.06	−18.72%	905.56	545.65%
MWD				
Gram negative	7.64	4.71%	1068.09	658.58%
Gram positive	6.82	4.11%	1008.94	607.94%
MT				
Gram negative	3.78	2.33%	1068.09	658.58%
Gram positive	−21.42	−12.91%	767.29	462.33%

¥Cfu/mL—Colony-forming units per milliliter, "−" Signifies reduction in bacterial cell count, "+" Signifies increase in bacterial cell count with absolute and relative change respectively.

6. Role of funding source

Research Project under grant number (R.G.P.1/159/40).

7. Ethical approval

(ECM#2019–107)—(HAPO-06-B-001).

8. Informed consent

Not Applicable.

CRediT authorship contribution statement

Snehil Dixit: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Writing - original draft, Writing - review & editing. **Irfan Ahmad:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing - original draft, Writing - review & editing. **Kumar Gular:** Conceptualization, Data curation. **Refaat A. Eid:** Formal analysis, Investigation, Writing - review & editing. **Ravi Shankar Reddy:** Methodology. **Ivana Leão Ribeiro:** Writing - original draft, Writing - review & editing. **Mohammed Abohashrh:** Project administration, Resources. **Mastour Saeed Alshahrani:** Software. **Jaya Shanker Tedla:** Investigation. **Nitin Arun Dixit:** Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors are thankful to the Deanship of Scientific Research, King Khalid University, Abha, Saudi Arabia, for financially supporting this work through General Research Project under grant number (R.G.P.1/159/40).

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