Journal of Pioneering Medical Sciences
Received January 18, 2024 Accept March 01, 2024 Publish July 30, 2024.
Volume 13, Issue 4, Pages 9-18

DOI https://doi.org/10.61091/jpms202413402

Photodynamic and Acoustic Therapy: A Breakthrough in Combating Antibiotic-Resistant Bacteria

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Abstract The emergence and spread of antibiotic-resistant bacteria is a major public health concern worldwide, posing a serious threat to the effectiveness of current antibiotic treatments. To address this issue, there is a growing need to explore alternative methods for controlling antibiotic-resistant pathogens. This study aims to provide a cost-effective and efficient process to reduce multidrug resistance and establish a system to eliminate multidrug-resistant bacteria. The study begins with the isolation of one or more bacteria in an aseptic, closed environment, followed by inoculation into saline. The sample is then exposed to either moderate sound wave at a predetermined frequency and intensity, red light at a predetermined wavelength, or both. The bacteria in the sample are exposed to the sound and red light, which may cause some of the resistant bacteria to become sensitive to antibiotics. The sound and red light are designed to penetrate the DNA of the bacteria, reversing their resistance to sensitivity. This process either promotes bacterial growth, causing them to scatter away from the canter of a petri dish, or kills them completely. In conclusion, When the organisms are exposed to audible sound alone or in combination with red light, colony formation decreases, and the colonies separate from each other, becoming weaker. In some cases, the colonies take on irregular shapes or die off considerably.

Key Words antibiotic, resistance, bacteria, light, sound wave

1. Introduction

Multidrug-resistant bacteria are becoming an increasingly serious problem in modern healthcare. The generalizability of much published research on this issue is problematic, as these bacteria have developed into highly resistant strains of multiple antibiotics and are responsible for a growing number of difficult-to-treat infections [1]. This is evident among gram-positive species such as Staphylococcus aureus, Enterococcus faecium, Enterococcus faecalis, and Streptococcus pneumoniae, and among gram-negative strains such as Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii [2], [3].

To overcome this problem, it is crucial to understand how bacteria communicate. According to conventional theory, bacteria are unicellular organisms that respond to environmental stimuli by detecting chemical and physical signals [4]. Bacteria communicate with one another via small 'hormone-like' chemical molecules known as autoinducers and through a process known as quorum sensing (QS), which is dependent on cell density and characterized by the coordinated release of signaling molecules that alter microbial metabolism and gene expression [5]. This case illustrates that gram-positive and gram-negative bacteria use different types of QS systems [6]. For example, Staphylococci bacteria use the accessory gene regulator (AGR) system to regulate the expression of genes encoding virulence factors, thereby coordinating the release of toxins and proteases necessary for colonization and adhesion [5]. Researchers have detailed four mechanisms leading to antimicrobial resistance: limiting drug uptake, modifying drug targets, inactivating drugs, and active drug efflux. An important example of a method to overcome antibiotic resistance is based on electromagnetic radiation with wavelengths ranging from 200 to 1,000 nanometers, known as Red Light Therapy (RLT). RLT is a treatment that may help in the healing of skin, muscular tissue, and other parts of the body by exposing the patient to red or near-infrared light at low intensity [7]. This case demonstrates the need for invention strategies like RLT to effectively combat antibiotic resistance [8].

Acinetobacter baumannii is a major public health concern in healthcare settings, particularly in critical ICU patients. This issue is highlighted by the exploration of alternative

treatment methods such as sound, red light, and photon therapy due to the bacteria's immunity to anti-infective drugs [9]. The authors challenge the prevailing view that the global medical and scientific community is increasingly interested in Acinetobacter baumannii for its multidrug resistance (MDR) and resistance to three or more distinct antibiotic classes, including last-resort antibiotics [10].

Extended-spectrum β-lactamases (ESBLs), including TEM, SHV, CTX-M, and GES enzymes, are plasmid-encoded and primarily found in Klebsiella species, Escherichia coli, and other Enterobacterales. These enzymes hydrolyze penicillin (e.g., piperacillin), most cephalosporins (cephamycins are not hydrolyzed by most ESBLs), and monobactams. However, the antibiotic susceptibility pattern of ESBLs, which is 4.8% and 15.8% in various parts of Saudi Arabia, is a significant public health concern. Public health agencies are critical of the new policies on multidrug resistance, as they have become a burden. Approximately 90% of community-acquired urinary tract infections are caused by E. coli, and some strains are characterized as uropathogenic bacteria, while K. pneumoniae causes lobar pneumonia, urinary tract infections, septicemia, and neonatal meningitis [11].

Prior research has shown that sound greatly affects the electromagnetic field of water molecules, influencing the consistency and vibration of water atoms in cells and, consequently, human recovery. Evidence supports the potential of phenothiazinium dyes, especially new methylene blue, as photosensitizers for photodynamic therapy (PDT) in treating burns caused by multidrug-resistant Acinetobacter baumannii. A thermal light source may generate photons with strong spatial correlations, enhancing bacterial photosynthesis. The effectiveness of the thermal light technique is exemplified in a report by Tegos et al. [12], which shows the organism's ability to utilize spatially correlated incoming light in membranes with high assembly of core complexes [12]. Therefore, there is a need for methods and systems to eliminate multidrug-resistant bacteria that can address the afore mentioned drawbacks while providing a cost-effective and efficient process to reduce resistance.

2. Materials and Methods

A. Study design

The steps of the study were summarized in Figure 1. Figure 1 illustrates a method for removing resistance in multidrug-resistant bacteria, in accordance with an embodiment of the present invention.

B. Isolation of one or more bacteria in an aseptic closed environment

. The bacteria are selected from a group comprising ESBL (Extended Spectrum β-Lactamases) Escherichia coli, ESBL Klebsiella pneumoniae, MRSA (Methicillin-Resistant Staphylococcus aureus), and multidrug-resistant Acinetobacter baumannii. The selected bacteria are collected under aseptic conditions from different infection sites of patients admitted to the ICU of King Abdulaziz University Hospital.

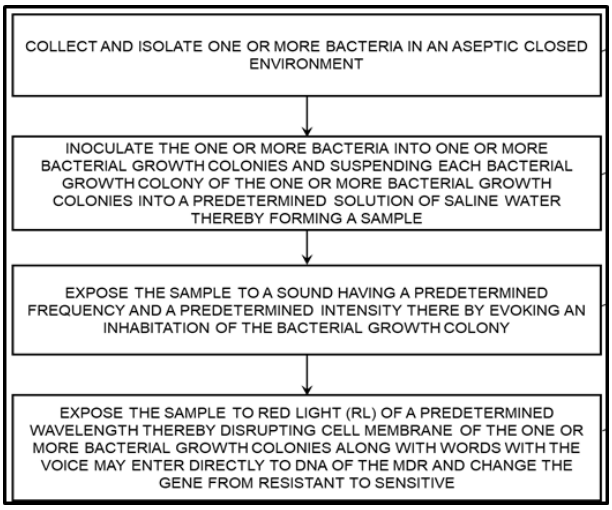

Figure 1: Experimental design

Seventeen MRSA samples were taken from different patients of various genders, ages, and infection sites to study MRSA and antibiotic resistance. The 17 patients with MRSA included 9 males and 8 females, with an average age of 54 years (2 males aged 54-84 years, and 5 females aged 53-80 years). Respiratory samples were taken from 3 males aged 31-58 years, including smear samples from them. Out of 48 ESBL samples, 18 were Klebsiella spp., with all except one identified as K. pneumoniae (one was K. oxytoca), and 30 samples were ESBL E. coli. Among these, 24 samples were from females (8 females aged 0-40 years) and 5 samples were from males aged 41-80 years. Additionally, 11 samples were from females and 6 were from males out of 29 samples. The samples were predominantly collected from females more than males.

C. Inoculating into one or more bacterial growth colonies and suspending into normal saline solution thereby forming a sample

The predetermined normal saline solution ranges from 0.1% to 0.9% w/v sodium chloride, with a dilution factor of 0.5 McFarland standard. The predetermined salt concentration may range from 10 g/L to 50 g/L. The salt used may be selected from a group comprising sodium chloride, potassium chloride, potassium sulphate, calcium chloride, magnesium sulphate, magnesium chloride, concentrated dairy minerals, and reduced sodium sea salts.

D. Exposing the sample to the recitation of moderate sound wave

Reciting verses from the moderate sound directly to a sample, using a voice with a specific vibration, pattern, and sound at predetermined frequencies and intensities, can inhibit bacterial colony growth. Certain phrases and words from the moderate Arabic sound wave are used to reduce bacterial resistance.

E. Exposing samples to near-infrared light (NIR) ranging from (600 to 1000 nm)

Exposing samples to near-infrared light (NIR) ranging from 600 to 1000 nm has shown promise in treating infections and aiding patient recovery, particularly when combined with red light. This combined therapy not only effectively kills bacteria but also supports the healing process of affected tissues. It is essential to administer any light therapy under proper medical guidance to mitigate potential adverse effects.

F. Near-Infrared Light (600-1000 nm)

- Penetration Depth: NIR light can penetrate deep into subcutaneous tissue and muscles.
- Effectiveness: NIR light is effective for treating deeper tissue infections and promotes tissue repair and healing.
- Mechanism: It enhances cellular functions, promotes circulation, increases mitochondrial activity, and exhibits mild antimicrobial effects.

G. Optimal Choice: Combination Approach

- Red and Near-Infrared Light (600-900 nm): This combination, commonly used in medical treatments, offers antibacterial effects and promotes tissue healing. Red light (around 660 nm) and NIR light (around 850 nm) are often used together in photobiomodulation therapy.
- Safety: Both types of light are generally safe for human use when applied at appropriate intensities and durations.

H. Photobiomodulation Therapy (PBMT)

- Uses: PBMT utilizes low-level lasers or light-emitting diodes (LEDs) to treat various conditions, including infections and wound healing.
- Benefits: PBMT reduces inflammation, accelerates tissue repair, enhances circulation, and exhibits antimicrobial properties.

In clinical settings, LED devices emitting specific wavelengths within these ranges are often employed. These devices can be adjusted for intensity and exposure duration to optimize therapeutic outcomes.

Using red and near-infrared light therapy (600-900 nm) in systemic diseases and critical patients can be beneficial, but its application must be carefully managed and tailored to individual patient needs and conditions. This type of therapy, known as photobiomodulation therapy (PBMT), has shown promise in various medical contexts:

I. Potential Benefits in Systemic Diseases and Critical Care

1) Anti-Inflammatory Effects: Red and NIR light can reduce systemic inflammation, beneficial for chronic and acute conditions like autoimmune diseases and sepsis.
2) Enhanced Immune Function: PBMT may modulate the immune response, potentially improving the body's ability to fight infections and manage systemic illnesses.
3) Tissue Repair and Healing: PBMT promotes cellular repair mechanisms, aiding in the recovery of damaged tissues critical for critically ill patients.
4) Pain Reduction: PBMT exhibits analgesic properties, useful for managing pain in critically ill patients without pharmacological side effects.
5) Improved Circulation: Enhancing blood flow and oxygenation through PBMT supports overall systemic health, particularly for patients with cardiovascular issues or those in critical care settings.

J. Considerations and Precautions

1) Medical Supervision: PBMT should always be administered under healthcare professional supervision, especially in critical care settings, to ensure safe and effective application.
2) Individualized Treatment: PBMT dosage, duration, and frequency should be tailored to each patient's specific needs, considering overall health, disease state, and concurrent treatments.
3) Contraindications: Certain conditions or medications may contraindicate PBMT use, requiring careful evaluation before treatment.
4) Monitoring and Adjustment: Continuous patient monitoring and treatment protocol adjustments are essential to optimize outcomes and manage potential side effects.

K. Clinical Applications and Evidence

- Wound Healing: PBMT is widely used for promoting chronic and acute wound healing, including in critically ill patients.
- Sepsis: Preliminary studies suggest PBMT may modulate immune responses and reduce inflammation in septic patients.
- Chronic Pain: PBMT offers a non-invasive pain management option for patients with chronic systemic conditions.
- Neurological Conditions: PBMT shows promise in treating neurodegenerative diseases and brain injuries, though further research is needed.

In summary, red and near-infrared light therapy can be a valuable adjunctive treatment in managing systemic diseases and critical patients when administered under appropriate medical supervision. Its benefits in reducing inflammation, enhancing immune function, promoting tissue repair, and improving circulation make it a versatile therapeutic option. However, personalized and closely monitored application is essential to ensure safety and efficacy.

L. Antimicrobial susceptibility test

Antimicrobial susceptibility testing is performed using the Kirby–Bauer disc-diffusion technique on Mueller–Hinton agar, following CLSI guidelines (2021). The antimicrobial discs used include Amoxicillin/Clavulanic Acid (20/10 μg), Cefoxitin (30 μg), Ceftazidime (30 μg), Ciprofloxacin (10

μg), Gentamicin (30 μg), Trimethoprim/Sulfamethoxazole (1.25/23.75 μg), and Clindamycin (2 μg). Freshly grown colonies are suspended in normal saline, and the turbidity of the suspension is adjusted to 0.5 McFarland's standard.

For the initial 40 samples from pure colonies on the original plate, they are exposed to a specific frequency sound identified from moderate sound wave and red light (RL) once. Subsequently, other samples adjusted to 0.5 McFarland's standard are exposed to SQC and RL three times. These suspensions are inoculated onto Mueller–Hinton agar using a sterile cotton swab, and all the antibiotic discs are placed 20 mm apart. Plates are incubated at 37°C for 24 hours.

After overnight incubation, the zones of inhibition around the discs are measured with a ruler and interpreted according to CLSI guidelines and VITEK 2 System criteria. Sensitivity thresholds are as follows:

- Cefoxitin: $\geq$ 22 mm
- Ceftazidime: $\geq$ 18 mm
- Ciprofloxacin: $\geq$ 26 mm (Enterobacterales), $\geq$ 21 mm (Staphylococcus)
- Trimethoprim/Sulfamethoxazole: $\geq$ 16 mm
- Gentamicin: $\geq$ 15 mm
- Clindamycin: $\geq$ 21 mm
- Amoxicillin/Clavulanic Acid: $\geq$ 18 mm

The experiment aims to evaluate the effects of SQC and RL on multidrug-resistant organisms. The organisms are exposed to SQC and RL individually and in combination. Due to time, cost constraints, and the novelty of the approach, the experiment is exploratory in nature.

3. Results

The light effect: Photodynamic treatment improved the vitality of ESBL-positive E. coli. Increased exposure duration to the photosensitizer enhanced the viability of ESBL-negative Klebsiella while decreasing the viability of ESBL-positive Klebsiella. In response to photodynamic treatment, ESBL-positive E. coli tends to deteriorate.

The sound effect: The response of Escherichia coli cells to audible sound stimulation was examined under both normal and stressful conditions. The results reveal that when E. coli is exposed to audible sound, colony formation rises considerably under normal growth conditions.

Table 1 showed that the zone of inhibition is measured by using a ruler and interpreted by comparing the VITEK 2 System according to the CLSI guidelines. The isolates with susceptibility to Cefoxitin (zone diameter of $\geq$ 22 mm), ceftazidime (zone diameter of $\geq$ 18 mm), Ciprofloxacin (zone diameter of $\geq$ 26 mm for Enterobacterales) and Ciprofloxacin (zone diameter of $\geq$ 21 mm for Staphylococcus), Trimethoprim/Sulfamethoxazole (zone diameter of $\geq$ 16 mm), Gentamycin (zone diameter of $\geq$ 15 mm), Clindamycin (zone diameter of $\geq$ 21 mm), and Amoxicillin/Clavulanic Acid (zone diameter of $\geq$ 18 mm), around the disks are suspected as sensitive (Table 1).

As shown in Table 2, multidrug-resistant (MDR) bacteria were randomly exposed to different treatments: moderate sound wave alone, red light (RL) alone, SQC and RL together, and all three combined. At least two antibiotics to which the bacteria were resistant in the initial sensitivity test were selected for re-testing after exposure to these treatments. Out of 17 MRSA samples, the following results were observed:

- Clindamycin: 2 samples became sensitive after exposure to SQC alone and SQC with RL.
- Trimethoprim/Sulfonamides: 1 sample showed significant sensitivity after exposure to SQC alone, RL alone, and SQC with RL.
- Oxacillin: 1 sample became sensitive after exposure to SQC alone, RL alone, and SQC with RL.

When Kefir and Kombucha probiotic bacteria were added to enhance the effect of SQC and RL, a synergistic effect was observed.

For MDR A. baumannii, clindamycin, ciprofloxacin, oxacillin, and trimethoprim/sulfonamides were chosen as the most resistant antibiotics for further testing after exposure to SQC and RL. The most frequently isolated bacteria were from the throat and respiratory tract, with an average patient age of 54.8 years. Out of nine antibiotics tested, Piperacillin/Tazobactam, Ceftazidime, Ciprofloxacin, and Trimethoprim/Sulfa showed resistance and were chosen for re-testing after SQC and RL exposure.

The effect of SQC alone or with RL was highly significant on MDR A. baumannii compared to other multi-resistant MRSA and ESBL organisms tested. Seven out of ten A. baumannii samples showed complete cell death when antibiotics were tested after exposure to SQC alone or with RL. The most effective treatments were observed with ciprofloxacin, followed by ceftazidime. To confirm these results, one sample was re-tested and showed consistent outcomes. Adding Kefir and Kombucha probiotic bacteria to one sample to enhance the effect of SQC and RL did not yield any additional improvement.

Of the 16 samples tested for antibiotic susceptibility against ESBL-positive Klebsiella pneumoniae isolated from KAUH, all samples were sensitive to Ciprofloxacin and resistant to all antibiotics except Meropenem, Imipenem, Ertapenem, and Amikacin. Four samples showed intermediate resistance to Ciprofloxacin and Nitrofurantoin. In the 48 samples tested against ESBL-positive Escherichia coli, four antibiotics were evaluated: Amoxicillin/Clavulanic Acid, Ciprofloxacin, Gentamicin, and Trimethoprim/Sulfa. Three conditions were studied: SQC alone (L), RL alone (S), and SQC with RL (L&S). The results for ESBL-positive Klebsiella pneumoniae before treatment indicated resistance to all antibiotics except Amoxicillin/Clavulanic Acid, which was sensitive. When Kombucha and Kefir were used against the ESBL bacteria, significant effects were observed. Out of the 16 samples taken from urine, respiratory, blood, and miscellaneous swabs, the most affected group were males aged 20 to 80 years. ESBL-producing Escherichia coli has seen a tremendous increase worldwide and is a common

Pipe/Taz	Amik	Amox/Clav	Cipro	Nitr	Gent	Trim/Sul	Etra	Mero	Imip
S	S	R	R	S	R	R	-	-	-
S	-	S	R	S	S	R	-	-	-
S	-	S	R	S	S	S	S	S	-
S	-	R	R	S	S	R	S	-	-
S	-	R	R	S	S	R	-	-	-
S	-	S	R	S	S	R	-	-	-
S	-	S	R	S	S	S	-	-	-
S	R	S	R	S	R	S	S	S	
S	S	S	S	S	R	S	S	S	-
S	S	S	R	S	R	R	S	S	-
S	-	S	R	S	S	R	S	S	-
S	-	S	S	S	S	S	S	S	-
S	-	S	R	S	S	S	-	S	-
R	-	R	R	S	S	R	-	S	S
S	S	R	R	S	R	R	S	S	-
S	-	S	R	S	S	R	S	S	-
S	-	S	R	S	S	R	-	-	-
S	S	R	R	S	R	R			
-	-	-	R	-	S	R	-	S	S
-	-	-	R	-	S	R	S	S	S
S	S	S	S	S	S	R	-	S	-
S	-	S	S	S	S	R	-	-	-
S	-	S	R	S	S	R	S	S	-
-	-	-	S	S	S	R	S	S	S
S	-	S	S	S	S	S	S	S	-
S	S	S	R	S	R	R	S	S	
S	-	S	S	S	S	R	-	-	-
S		S	S	S	S	S	-	-	-
S	-	S	R	S	S	R	S	S	-

Table 1: The susceptibility test of several antibiotic against ESBL positive Escherichia coli

cause of morbidity and mortality associated with hospital-acquired infections.

There is a strong association between multidrug resistance and ESBL-producing isolates. The present study aimed to determine the antimicrobial sensitivity profile of ESBL-producing E. coli isolates from various clinical samples. The effects of SQC, alone or combined with RL, on ESBL-producing E. coli and K. pneumoniae demonstrated potent sensitivity to two previously resistant antibiotics. The susceptibility test results showed that Ciprofloxacin, which was resistant before treatment, became sensitive in about half of the ESBL organisms after treatment. Trimethoprim/Sulfonamides also appeared more sensitive after treatment, though less so than Ciprofloxacin and Amoxicillin/Clavulanic Acid. The response of ESBL-producing Escherichia coli and Klebsiella pneumoniae and K. oxytoca cells to audible sound stimulation of moderate sound recitation, alone or combined with RL, showed improved antibiotic sensitivity. SQC improved the vitality of E. coli that was ESBL-positive. Prolonged exposure to SQC with RL increased the viability of ESBL-negative Klebsiella while decreasing the viability of ESBL-positive Klebsiella. E. coli showed a decline in response to these treatments. In summary, the study demonstrated that moderate sound wave and red light (RL) therapies could enhance the sensitivity of multidrug-resistant ESBL-producing bacteria to antibiotics, with notable improvements observed in E. coli and Klebsiella pneumoniae. This innovative approach could offer a novel means of combating antibiotic resistance in clinical settings.

antibiotics.

As shown in figure 2, significant differences ($P<0.001$) in biomass were observed when E. coli K-12 is exposed to sound frequency 2 kHz and 8 kHz, which may be increased by about 21.04% and 27.06% versus the control group, respectively. Meanwhile, exposure of E. coli K-12 to 2 kHz and 8 kHz sound waves also lead to an increase of the μmax, reflecting a faster growth of the treated group than the control group. Also, the average length of E. coli cells increased more than 29.67%. The maximum biomass and maximum specific growth rate of the stimulation group by 7900 Hz, 90dB sound wave was about 1.8 times and 3.4 times that of the control group, respectively. Moreover, E. coli respond rapidly to sound stress at both the transcriptional and posttranscriptional levels by promoting the synthesis of intracellular RNA and total protein. Therefore, it is suggested that some potential mechanisms may be involved in the responses of bacterial cells to sound stress. As shown in Figure 3, effect of sound Intensity levels (sound intensity level varied from 0 to 100 dB and maintained sound frequency 8 KHz and sound power level 55 dB) on growth of E. coli. In addition, Figure 4 illustrates effects of sound power level on the growth of E. coli, effect on growth of E. coli by sound fields (sound power level varied from 55 to 63 dB and maintained sound frequency 8KHz and sound intensity level 80 dB). As shown in figure 5, the total intracellular protein and RNA of E. coli exposed to sound wave at different time. (A) The total intracellular protein. The cells exposed to sound frequency 8KHz, intensity level 100 dB and power level 61dB.

	ESBL	Antibiotic	Before treatment	After treatment				
			Resistant	SCQ	RL	QSC+RL	Kamboucha QSC RL QSC&RL	KEFIR QSC RL QSC&RL
1	ESBLE. coli	Ciprofloxacin	R	S	-	S	-	-
2	ESBLE. coli	Ciprofloxacin	R	R	-	R	-	-
		Trimethroprim/Sulfa	R	R	-	R		
3	ESBLE. coli	Ciprofloxacin	R	R	-	R	-	-
		Trimethroprim/Sulfa	R	R	-	R		
4	ESBLE. coli	Ciprofloxacin	R	R	-	R	-	-
		Trimethroprim/Sulfa	R	R	-	R		
5	ESBL K. pneumoniae	Ciprofloxacin	R	R	-	S*	-	-
		Trimethroprim/Sulfa	R	R	-	R		
6	ESBL K. pneumonia	Ciprofloxacin	R	S	-	-	-	-
		Trimethroprim/Sulfa	R	R	-	-	-	-
7	ESBL K. pneumonia	Ciprofloxacin	R	S	-	S*		
		Trimethroprim/Sulfa	R	R	-	R-	-	-
8	ESBLE. coli	Ciprofloxacin	R	S	-	-		
		Trimethroprim/Sulfa	R	R	-	-		
9	ESBLE. coli	Trimethroprim/Sulfa	R	R	-	R	-	-
10	ESBL K. oxytoca	Ciprofloxacin	R	R	-	R-	-	-
		Trimethroprim/Sulfa	R	R	-	R	-	-
11	ESBL K. pneumonia	Ciprofloxacin	R	R	-	S	-	-
12	ESBL K. pneumonia	Ciprofloxacin	R	R	-	R-	-	-
		Amoxicillin/Clavulanic Acid	R	R	-	R	-	-
13	ESBLE. coli	Ciprofloxacin	R	R	-	I^	-	-
		Trimethroprim/Sulfa	R	R	-	R	-	-
14	ESBLE. coli	Ciprofloxacin	R	R	-	R	-	-
		Amoxicillin/Clavulanic Acid	R	R	-	R	-	-
15	ESBLE. coli	Ciprofloxacin	R	S	R	-	-	-
		Trimethroprim/Sulfa	R	S	R	-	-	-
16	ESBLE. coli	Ciprofloxacin	R	R	-	R	-	-
		Trimethroprim/Sulfa	R	R	-	R	-	-
17	ESBL K. pneumonia	Ciprofloxacin	R	-	-	S&	-	-
		Trimethroprim/Sulfa	R	-	-	R&	-	-
18	ESBLE. coli	Ciprofloxacin	R	-	-	S****	-	-
		Trimethroprim/Sulfa	R	-	-	S****	-	-
19	ESBLE. coli	Ciprofloxacin	R	-	-	S	-	-
		Trimethroprim/Sulfa	R	-	-	R	-	-
20	ESBL K. pneumonia	Ciprofloxacin	R	-	-	R	-	-
21	ESBLE. coli	Ciprofloxacin	R	-	-	S	-	-
22	ESBLE. coli	Ciprofloxain	R	-	-	R***	-	-
		Trimethroprim/Sulfa	R	-	-	R***		
23	ESBL E. coli	Gentamicin	R	-	-	R		
24	ESBL E. coli	Ciprofloxacin	R	-	-	R		
25	ESBLE. coli	Ciprofloxacin	R	-	-	S***		
26	ESBL K. pneumonia	Trimethroprim/Sulfa	R	-	-	S* SIGNIFICANT	-	-
27	ESBL E. coli	Ciprofloxacin	S	-	-	SS	-	-
28	ESBL K. pneumonia	Ciprofloxacin	R	-	-	S*****	-	-
		Trimethroprim/Sulfa	R	-	-	S*****		
29	ESBLE. coli	Ciprofloxacin	R	-	-	S*****	-	-
		Trimethroprim/Sulfa	R	-	-	S*****		
30	ESBL E. coli	Ciprofloxacin	R	-	-	R***	-	-
		Trimethroprim/Sulfa	R	-	-	R***		
31	ESBL E. coli	Ciprofloxacin	R	-	-	R		
		Trimethroprim/Sulfa	R	-	-	R		
32	ESBL K. pneumonia	Ciprofloxacin	R	-	-	S*	-	-
		Trimethroprim/Sulfa	R	-	-	S*		
33	ESBLE. coli	Trimethroprim/Sulfa	R	-	-	R		
34	ESBL K. pneumonia	Trimethroprim/Sulfa	R	-	-	R		
35	ESBL E. coli	Ciprofloxacin	R	-	-	S*****	- - R	- - R
		Trimethroprim/Sulfa	R	-	-	S*****	R	R
36	ESBLE. coli	Amoxicillin/Clavulanic Acid	S	-	-	SS	-	-
		Trimethroprim/Sulfa	S	-	-	SS		
37	ESBL K. pneumonia*	Ciprofloxacin	R	R&	R&	R&	R R R	R R R
		Trimethroprim/Sulfa	R	R*&	R*&	R*&	R R R	R R R
38	ESBL K. pneumoniae	Ciprofloxacin	R	R	R	R	-	-
		Gentamicin	R	R	R	R	-	-
39	ESBL K. pneumonia*	Ciprofloxacin	R	S*	S*	S*	-	-
		Trimethroprim/Sulfa	R	S*	S**	S**		
40	ESBL K. pneumoniae	Ciprofloxacin	S	S	S	S	-	-
		Trimethroprim/Sulfa	R	R	R	R		
41	ESBLE. coli	Trimethroprim/Sulfa	R	S*	S*	S*		
42	ESBL K. pneumonia	Ciprofloxacin	R	I	I	I	-	-
		Trimethroprim/Sulfa	R	R*	R*	R*		
43	ESBLE. coli	Ciprofloxacin (CONTROL)	S	S	S	S	-	-
		Trimethroprim/Sulfa (CONTROL)	S	S#	S#	S#		
44	ESBLE. coli	Trimethroprim/Sulfa	R	R*	R*	R*		
45	ESBL K. pneumonia	Ciprofloxacin	I	S#	S#	S#	-	-
		Trimethroprim/Sulfa	R	R*	R*	R*		
46	ESBLE. coli	Ciprofloxacin	R	R	R	R	-	-
		Trimethroprim/Sulfa	R	S	S	S		
47	ESBLE. coli	Ciprofloxacin	R	R	R	R	-	-
		Trimethroprim/Sulfa	R	R*	R*	R*		
48	ESBL K. pneumonia	Ciprofloxacin	R	I	I	I	-	-
		Trimethroprim/Sulfa	R	R*	R*	R*		

Table 2: The effect of frequency of word and red light separately or in combined against the ESBL positive Escherichia coli bacteria which showed previously resistant of two selected

		Antibiotic	Before treatment	After treatment				
			Resistant	S	L	S+L	Kamboucha S L S&L	Kefir S L S&L
1	MDR A. baumannii	1-Piperacillin\Tazobactam	R	S*	-	S*	-	-
		2-Ceftazidime	R	S*	-	S*	-	-
2	MDR A. baumannii	1-Piperacillin\Tazobactam	R	S*	-	S*	-	-
		2-Ceftazidime	R	S*	-	S*	-	-
3	MDR A. baumannii	1-Ciprofloxacin	R	S*	-	S*	-	-
		2-Ceftazidime	R	S*	-	S*	-	-
4	MDR A. baumannii	1-Ciprofloxacin	R	S*	-	S*	-	-
		2-Ceftazidime	R	S*	-	S*	-	-
5	MDR A. baumannii	1-Ciprofloxacin	R	-	-	R	-	-
		2-Trimethroprim\Sulfa	R	-	-	R	-	-
6	MDR A. baumannii	1-Ciprofloxacin	R	-	-	R	-	-
		2-Trimethroprim\Sulfa	R	-	-	R	-	-
7	MDR A. baumannii	1-Piperacillin\Tazobactam	R	-	-	S*&	-	-
		2-Ciprofloxacin	R	-	-	S*&	-	-
8	MDR A. baumannii	1-Ciprofloxacin	R	-	-	S**	R*** - -	R*** - -
		2-Trimethroprim\Sulfa	R	-	-	S**	-	-
9	MDR A. baumannii	1-Ciprofloxacin	R	-	-	R***	-	-
		2-Trimethroprim\Sulfa	R	-	-	R***	-	-
10	MDR A. baumannii	1-Ciprofloxacin	R	S*	R*	R*	R* R* R*	R* R* R*
		2-Trimethroprim\Sulfa	R	S*	R*	R*	R* R* R*	R* R* R*

Table 3: The effect of frequency of word and red light separately or in combined against the MRSA bacteria which showed previously resistant of two selected antibiotics

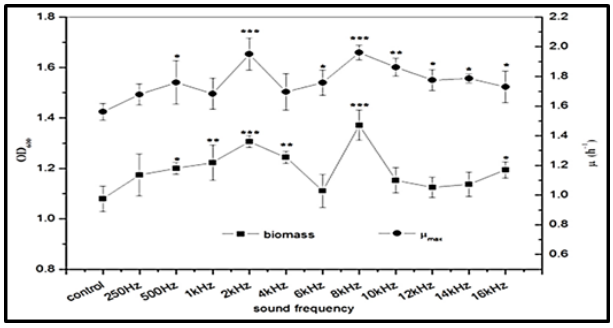

Figure 2: Illustrates Effect of different acoustic parameters on the growth of E. coli

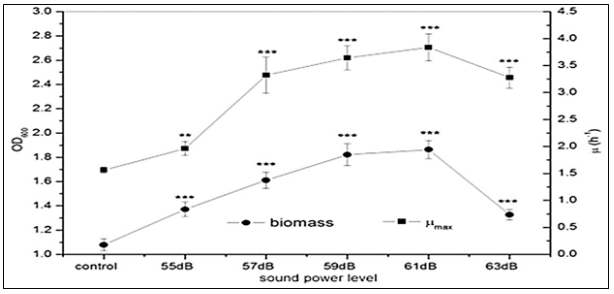

Figure 4: Illustrates effects of sound power level on the growth of E. coli,

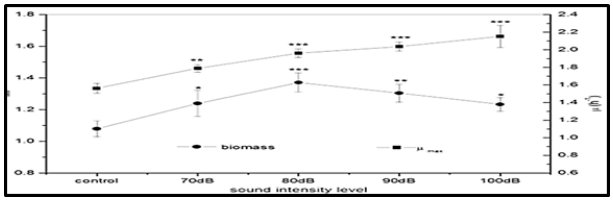

Figure 3: Illustrates the effects of sound intensity level on the growth of E. coli, in accordance with an embodiment of the present invention.

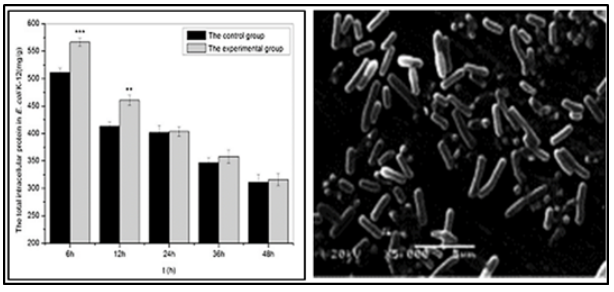

Figure 5: . Illustrates effect of sound on intracellular protein and RNA of E. coli,

Figures 6 illustrate phenomenon of growth which scattered one or more bacteria away from center of a petri dish. The bacteria in the sample are adapted to hear the sound words and the sound along with the red light make some of resistant bacteria become sensitive to antibiotics. The sound and the red light are adapted to penetrate DNA gene and reverse it from resistant to sensitive which shows a phenomenon of growth which scattered the bacteria away from center of a petri dish exposed with the sound and the red light or killed them completely.

The comparison of the bacterial growth before and after exposure to the sound and the red light are illustrated in Figure 7.

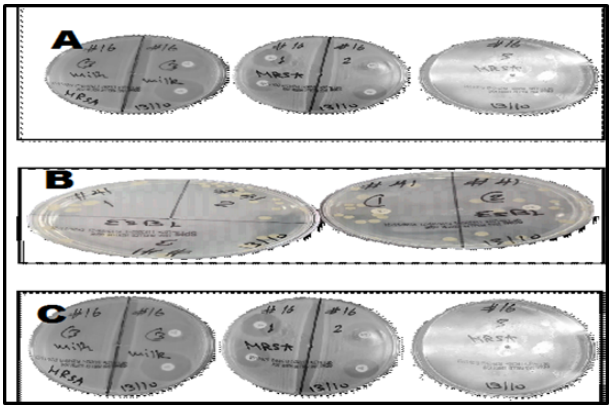

Figure 6: Illustrate phenomenon of growth which scattered one or more bacteria away from center of a petri dish.

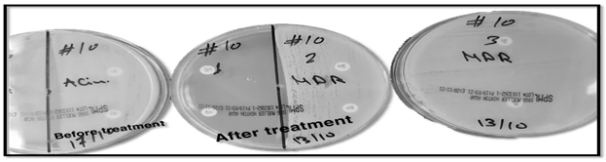

Figure 7: Illustrates before and after exposure to the sound and the red light.

4. Discussion

The present study revealed that bacteria can adapt to auditory stimuli from moderate sound wave and, when combined with red light (RL), some antibiotic-resistant bacteria become sensitive to antibiotics. This effect is achieved by disrupting the cell membranes of one or more bacterial colonies. These results are consistent with the findings of Hamblin and Abrahamse [8], who highlighted various applications of light in combating drug-resistant pathogens, and Gu et al. [13], who showed that sound could penetrate bacterial DNA and reverse antibiotic resistance. Specifically, the bacteria showed altered growth patterns, moving away from the center of a petri dish exposed to QSC and RL, or dying completely.

In the study, certain moderate sound, when recited directly to bacterial samples using specific vibrations, patterns, frequencies, and intensities, created mechanical stress. Subsequent susceptibility tests indicated that some bacteria became more sensitive to antibiotics. This phenomenon supports the findings of Gu et al. [13], who documented that sound is sensed by intracellular growth vessels, prompting microorganisms, including bacteria, to respond rapidly to stress at both transcriptional and post-transcriptional levels. The results suggest that bacteria, in addition to communicating through Quorum Sensing (QS), can adapt to auditory stimuli. Direct recitation of moderate sound wave to bacterial samples had similar effects to antibiotics in susceptibility tests, showing that the bacteria became sensitive and lost their resistance. Photodynamic treatment showed improved vitality of ESBL-positive E. coli, with increased exposure to the photosensitizer decreasing ESBL-positive Klebsiella viability while increasing ESBL-negative Klebsiella viability. E. coli tended to deteriorate in response to photodynamic treatment. Additionally, audible sound stimulation significantly increased E. coli colony formation under normal growth conditions, consistent with the findings of Al-Sarraj [14].

Jonas et al. [15] reported that high-intensity sound waves could disrupt bacterial cell walls, leading to destruction through sonoporation, which involves the formation of small pores in cell membranes using ultrasound for nucleic acid transfer. Low-intensity sound waves, however, might enhance microbial cellular metabolism, promoting growth and reproduction. This study demonstrates that bacteria possess an auditory system that allows them to "hear" and respond to moderate sound wave. Specific sound wave, especially chosen Ayat, and particular sounds can effectively disrupt or deactivate bacterial growth, offering a novel approach to managing antibiotic resistance.

In attempt to apply the results of the current study, the study recommends creating a disclosed system to remove resistance of multidrug resistant bacteria. The system is illustrated in Figure 8:

- The metal case may be a cubical or cuboidal metal box made of, but not limited to, steel, copper, metal alloys etc, configured to receive a sample within the beaker with the magnetic stirrer in the metal case. Further, the metal case is configured to encloses the beaker and the speaker, and the sound-absorbing material is used to reduce noise and interference, and the magnetic stirrer is placed within the beaker to agitate the sample.
- A metal case having a sound absorbing material inside, configured to receive a sample within a beaker with a magnetic stirrer.
- The beaker is made of a material that is transparent to ultraviolet radiation.
- The sound absorbing material not limited to, cellulose, aerated plaster, fibrous mineral wool and glass fiber, open-cell foam, and felted or cast porous ceiling tile
- The magnetic stirrer may be adapted to constantly mix the sample.
- A sound waves source configured to generate high-frequency sound waves at a predetermined frequency; It may be placed inside the metal case. It may be but is not limited to, a tuner, and microphone, an amplifier and/or a noise filter. The sound with different words with direct voice raveling through the suspension created a mechanical stress on the sample. The predetermined frequency is in range of 0Hz to 16kHz, and preferably in range of 200Hz to 300Hz and the sound intensity in range of 0dB -100dB.
- A sound waves transmission conductor, configured to transmits the sound waves from source into the metal case. It may be metal cable such as copper, aluminum or aluminum cable or optic fiber.
- A speaker, connected with the sound waves transmission conductor, configured to receive the sound waves and transform into a sound having the predetermined

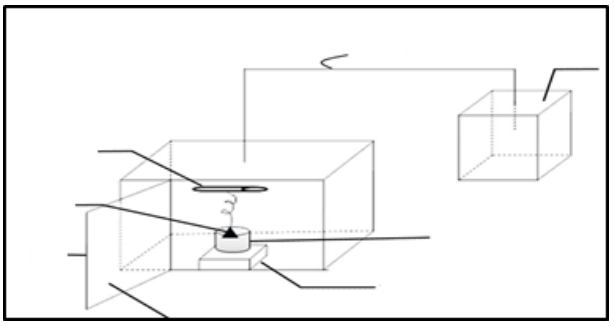

Figure 8: Illustrates a system to remove resistance of multidrug resistant bacteria, in accordance with an embodiment of the present invention.

frequency and a predetermined pattern to evoke an inhabitation of the bacterial growth colony wherein the sound comprises reciting words of moderate sound straight to the sample; the sound is sensed by a growth vessel inside the cell, and living microorganisms including microbes which rapidly respond to the stress at both transcriptional and post transcriptional level

- A light source configured to expose the sample to Red Light (RL) of a predetermined wavelength thereby disrupting cell membrane of the one or more bacterial growth colonies. The red-light exposure adapted to suppress the growth of bacterial microorganism and/or penetrate the bacterial gene and modified from resistant to sensitive.

The bacteria in the sample have adapted to hear sound words, and when combined with red light (RL), some antibiotic-resistant bacteria become sensitive to antibiotics. In addition to their existing communication via Quorum Sensing (QS), these bacteria utilize an auditory system to respond to sound.

Sound frequency is emerging as a new method to combat multidrug-resistant bacteria—those resistant to at least two or more classes of antibiotics. This study provides evidence supporting the development of an innovative sound frequency device. Such a device could potentially transform resistant bacteria into sensitive strains, making them susceptible to various antimicrobial drugs. Biofilms, which are colonies of microorganisms that form on surfaces, can be particularly difficult to eradicate. Sound therapy has been shown to reduce biofilm formation. Additionally, red light therapy (RLT) may boost the production of adenosine triphosphate (ATP) in cells, enhancing their energy production. This increased energy can be beneficial for microorganisms that require it for growth and reproduction. Furthermore, RLT may reduce inflammation in cells, which could be advantageous for treating microorganisms that cause inflammatory conditions. The effectiveness of sound therapy and RLT on microorganisms depends on several factors, including the intensity and duration of treatment, as well as the type of microorganism targeted [16].

5. Conclusion

The study reveals that when organisms are exposed to moderate sound wave alone or in combination with red light (RL), there is a noticeable decrease in colony formation. The colonies tend to separate from each other, become weaker, take on irregular shapes, and sometimes die off significantly.

6. Recommendation

Further studies are needed to refine this method and develop equipment that utilizes QSC and RL frequencies effectively.

Acknowledgment

Author is thankful to Faculty of science, King Abdulaziz University, Jeddah, Saudi Arabia, for technical and scientific supports.

Conflict of interest

Author declares no conflict of interests. All authors read and approved final version of the paper.

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