Journal of Pioneering Medical Sciences
Received May 15, 2024 Accept July 08, 2024 Publish July 30, 2024.
Volume 13, Issue 4, Pages 173-179

DOI https://doi.org/10.61091/jpms202413426

Investigates the Effects of the Sound Waves on the Growth and Antibiotic Susceptibility of Extended-Spectrum Beta-Lactamase (Esbl)-Producing E. Coli

Rania Mohammad Sabri Sultan[1,*]
[1]Faculty of science, King Abdulaziz University, Jeddah, Saudi Arabia.

Corresponding author: Rania Mohammad Sabri Sultan (e-mail: rmsultan@kau.edu.sa).

Abstract Antibiotic-resistant microbes have been closely associated with drug misuse and careless drug disposal, indicating a mutualistic relationship between certain environmental conditions and antibiotic resistance. This study investigates the effects of the sound on the growth and antibiotic susceptibility of ESBL-producing E. coli (ESBL-E. coli). We examined how various frequencies, intensities, and powers of sound exposure influenced E. coli development and antibiotic resistance. Our findings revealed that E. coli exposed to sound waves exhibited a more rapid specific growth rate and higher biomass compared to the control group. Specifically, the average length of E. coli cells increased by over 27.26%. The stimulation group experienced maximum biomass and specific growth rates approximately 1.7 and 2.5 times higher than the control group when exposed to an 8000 Hz, 80 dB sound wave. Before receiving sound treatment, ESBL-E. coli showed resistance to four antibiotics: amoxicillin/clavulanic acid, trimethoprim/sulfonamides, ciprofloxacin, and gentamicin. However, post-treatment susceptibility tests indicated that these antibiotics became effective. The study demonstrates that the moderate sound wave significantly impacts multidrug-resistant ESBL-E. coli. Attempts to enhance the sound effect using probiotic bacteria from kefir and kombucha showed no improvement. Notably, certain Moderate sound verses had a profound impact on E. coli, suggesting a unique approach to combating antibiotic resistance. Considering projections that effective antibiotics might be scarce between 2030 and 2050, this study offers a promising alternative. This groundbreaking research holds significant potential for application in Saudi Arabia and globally. We have secured a patent for this innovation from the United States of America, underscoring its importance and potential impact.

Key Words moderate sound wave, ESBL-E. coli, antibiotic-resistance, frequency, intensity

1. Introduction

Antimicrobial resistance has recently increased significantly, presenting a dynamic public health challenge that affects all genders and age groups. It is now a leading cause of death from infectious diseases globally, particularly concerning bacteria resistant to last-line antibiotics, suggesting a future with untreatable infections. Extensive research has focused on 30-day mortality rates of 15–20%, but no single study reports significantly higher rates than infections caused by methicillin-resistant Staphylococcus aureus (MRSA), carbapenem-resistant organisms (CRE), and Clostridium difficile infection [1]–[3]. Until recently, healthcare-associated E. coli bacteremia, an infection prevention and control (IPC) area, has been neglected. E. coli, part of the Enterobacteriaceae family, is the most common cause of urinary tract infections (UTIs), responsible for up to 80% of cases [4], [5]. Over the past 20 years, extensive use of antibiotics to treat pathogenic bacteria has led to widespread antimicrobial resistance, now a radical risk to global health [6].

Sound travels as a mechanical vibration in elastic media like liquid, air, and solids. In humans, the ability to perceive sound at different frequencies decreases with age. Humans can hear frequencies between 20 Hz and 20 kHz [7], while microorganisms are affected by vibrations outside this range, such as infrasound and ultrasound [8]. Vibrations can significantly impact the growth of both gram-positive and gram-negative bacteria, depending on the organism and the sound frequencies used. Studies have shown that sound frequencies between 1 and 5 kHz significantly increased growth in the bacterial model organism E. coli compared to quiet conditions [9]. However, more research is needed on the effects of sound intensity, as only E. coli has been

subjected to a range of sound intensities [10]. The Murottal Al-Qur'an generates sound through atmospheric oscillations or waves, with frequency defined as the number of vibrations or waves per second. Studies on the impact of Al-Qur'an sound on bacterial proliferation and antibiotic resistance are still incomplete. Consequently, this investigation will explore how short-term exposure to Al-Qur'an sound affects the development of pathogenic E. coli from various clinical samples. The goal is to deeply understand the antibiotic susceptibility profile of ESBL-producing E. coli isolates from different clinical samples before and after treatment, which is crucial for improving control and treatment outcomes.

2. Materials and Methods

A. Study design and sampling

The current study included patients in the intensive care unit at King Faisal Hospital who had been hospitalized for a minimum of 48 hours. Consequently, a census sampling approach was used, encompassing all 48 eligible patients from the Electronic Medical Record (EMR). The study involved a randomized experimental investigation by the Microbiology Lab, focusing on ESBL-producing E. coli strains found in various clinical samples. Specimens with more than 10^5 CFU/mL of specific ESBL-producing E. coli strains were cultured for this research.

B. Antibiotics susceptibility test pattern

The Kirby-Bauer disc-diffusion technique was used to test the following antimicrobial discs on Mueller-Hinton agar according to CLSI guidelines 2021: 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 were suspended in saline to adjust the turbidity to 0.5 McFarland standard. On average, forty pure colony samples were subjected to the detected frequency sound on the original plate. The remaining samples were adjusted to 0.5 McFarland standard and exposed to the determined frequency sound three more times. This suspension was then inoculated onto Mueller-Hinton agar using a sterile cotton swab. Antibiotic discs of Ciprofloxacin, Trimethoprim/Sulfamethoxazole, Oxacillin, Clindamycin, Gentamicin, Amoxicillin/Clavulanic Acid, and Ceftazidime were placed with a 20 mm spacing and incubated at 37°C for 24 hours. The zones of inhibition were measured with a ruler and interpreted by comparing the results with the VITEK 2 System according to CLSI recommendations.

C. Effect of sound of Moderate sound of different frequencies on growth and antibiotic sensitivity pattern

In the experimental setup, sound exposure tests were conducted (refer to Figure 1) to investigate the impact of sound recitations on the development and antibiotic sensitivity pattern of ESBL-producing E. coli (ESBL-E. coli). The study involved playing a sound for multidrug-resistant ESBL-E. coli. Following exposure to specific sounds, at least two drugs to which the bacteria had shown resistance in the sensitivity test were selected, and their sensitivity was retested.

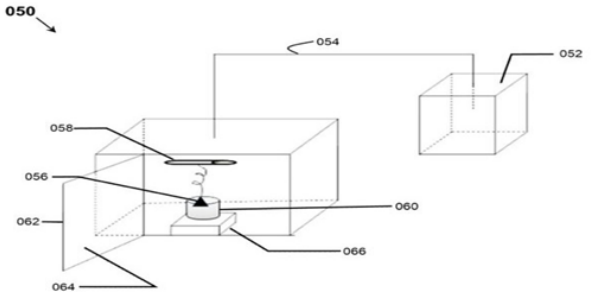

Figure 1: A system (050) designed to eliminate resistance in multidrug-resistant bacteria, embodying the present invention. The system (050) may include, but is not limited to, a metal case (062) containing a sound-absorbing material (064), a beaker (060) equipped with a magnetic stirrer (066), a source of sound waves (052), a conductor for transmitting sound waves (054), a speaker (056) connected to the sound waves transmission conductor (054), and a light source (058). As part of the present invention, the metal case (062) incorporates a sound-absorbing material (064) which may comprise cellulose, aerated plaster, fibrous mineral wool and glass fiber, open-cell foam, or felted or cast porous ceiling tile

D. Sound exposure experiments

E. coli samples were subjected to the following experimental conditions: (I) varying sound power levels between 55 and 63 dB, maintaining a frequency of 8 kHz and intensity of 80 dB; (II) varying sound intensity levels between 0 and 100 dB, maintaining a frequency of 8 kHz and power level of 55 dB; (III) varying sound frequencies between 250 and 16,000 Hz, maintaining intensity levels of 80 dB and 55 dB. The waveform generator and amplifying circuit of the sound-wave-producing unit adjusted the sound frequency and intensity levels accordingly. Variations in the size of the speaker were implemented to achieve different sound power levels. Samples not exposed to sound were designated as the control group. The temperature was maintained at 37 ± 1 °C throughout the experiment using the sound waves load equipment. Sound exposure occurred continuously during the experiment, and a magnetic stirrer was utilized to stir the samples for five minutes every 15 minutes.

E. Measurement of biomass and specific growth rate

The maximum optical density served as a measure of E. coli biomass, with the optical density of the culture broth determined at 600 nm. Cell dry weight was assessed through a six-hour drying procedure at 70°C.

F. Measurement of E. coli intracellular protein

After sampling every 6 hours, the culture was either diluted or concentrated to an optical density of 1 at 600 nm (OD600). Protein extraction was performed using a bacterial protein extraction kit, and the protein content was measured using a modified BCA protein assay kit.

G. Morphologic observation of E. coli

E. coli was exposed to sound waves at intensities of 80 dB and 100 dB, with a frequency of 8 kHz and a power level of 61 dB, respectively. Following a 48-hour sampling period, the cells were centrifuged, washed with distilled water, and dehydrated using graded ethanol (20%, 50%, 80%, and 100%). The dehydrated cells were then dissolved in alcohol. Glass slide samples were dried, and a layer of metal film was deposited on the surface using a vacuum evaporator. The morphology of E. coli was observed using a scanning electron microscope (SEM).

H. Statistical Analysis

To minimize variability, each experiment was conducted in triplicate. Data were presented as mean ± standard deviation and compared using a non-parametric Mann-Whitney test.

3. Results

This study comprised 48 patients admitted to the ICU for at least 48 hours, with clinical specimens primarily collected from the throat and respiratory tract. Among these patients, 29 (60.4%) were females, and 19 (39.6%) were males, with an average age of 54.8 years. Among the 48 samples analyzed, 30 (62.5%) tested positive for ESBL-producing E. coli (ESBL-E. coli).

A. Antibiotics susceptibility profile of ESBL-E. coli

Among the ten antibiotics tested, Trimethroprim Sulfa, Ciprofloxacin, Amoxicillin/Clavulanic Acid, and Gentamicin demonstrated resistance to ESBL-producing E. coli and were selected for further testing after moderate sound trating (Table 1).

B. Effects of sound citation on ESBL-E. coli growth

1) Sound Frequency effect The Wave Pad Sound Editor Masters Edition v 5.5 was used to analyze the frequency of the sound sound played. When subjected to sound frequencies of 2 kHz and 8 kHz, E. coli exhibited a significant increase in biomass, with levels rising by approximately 21.04% and 27.06% compared to the control group, respectively (P<0.001). Additionally, E. coli exposed to sound waves at frequencies of 2 kHz and 8 kHz demonstrated an increase in μmax, indicating accelerated growth compared to the control group (Figure 2).
2) Sound intensity level effect MODERATE SOUND waves with varying degrees of sound intensity were applied to E. coli at a frequency of 8 kHz and a power level of 55 dB. When comparing the treated group with an 80 dB sound intensity level to the control group, we observed a significant increase in ESBL-E. Coli biomass. The treated group initially showed a rapid increase in biomass, reaching a maximum of 1.371 (OD600) at a sound intensity level of 80 dB, approximately 27.06

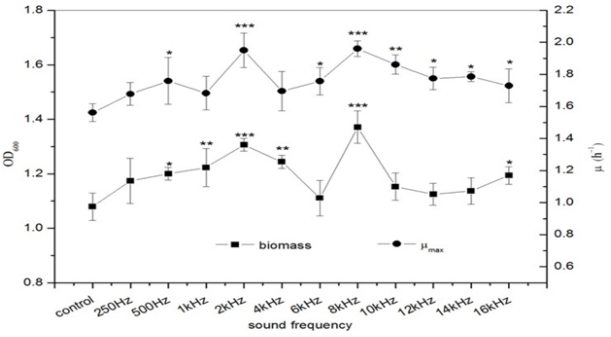

Figure 2: Effects of sound frequency on the growth of E. coli

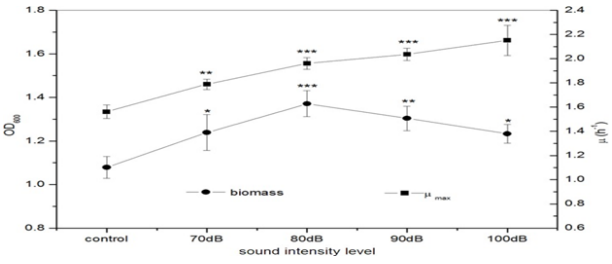

Figure 3: Effects of moderate sound intensity level on the growth of E. coli

3) Sound power level The growth of E. coli exposed to varying MODERATE SOUND power significantly increased, as demonstrated in Figure **??**. The biomass exhibited a roughly linear rise as sound strength increased, peaking at 59 dB. In contrast, the biomass gradually increased from 59 dB to 61 dB before drastically declining. The highest biomass of E. coli exposed to 61 dB of sound pressure was 1.863 (OD600), approximately 1.7 times higher than the biomass of the control group (OD600 1.079). E. coli's μmax increased rapidly, reaching a peak at 61 dB before dramatically dropping. Both biomass and μmax gradually decreased when the sound power level surpassed 61 dB, indicating that excessive sound exposure may inhibit E. coli growth through several mechanisms.
4) Effects of sound exposure on intracellular protein synthesis in E. coli Studies on the effects of MODERATE SOUND TREATMENT at frequencies of 8 kHz, intensity levels of 80 dB, and power levels of 61 dB on the intracellular macromolecules of E. coli revealed that specific sound exposures significantly impacted intracellular protein levels in E. coli. Both the treatment and control groups showed a gradual decrease in intracellular protein over time. However, after six hours of sound

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 antibiotics against ESBL-E. coli

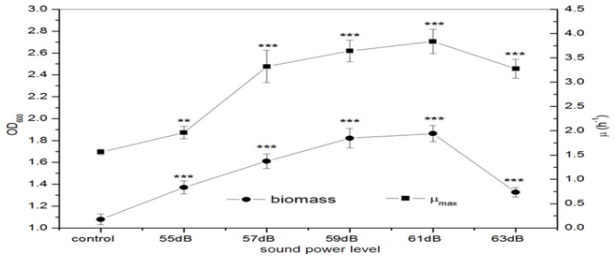

Figure 4: Effects of moderate sound power level on the growth of E. coli

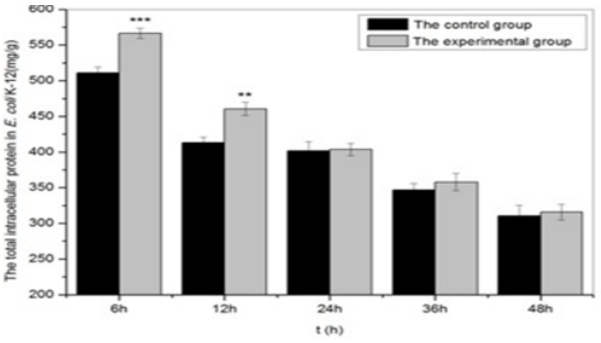

Figure 5: The total intracellular protein of E. coli exposed to sound waves at different times

exposure, the treatment group's intracellular protein concentration rose notably to 566.4 mg/g, nearly 1.1 times higher than the control group's 511.1 mg/g. This suggests that early stages of MODERATE SOUND TREATMENT exposure may significantly enhance intracellular protein production in E. coli, promoting cell proliferation (Figure 5).

C. Morphological change of E. coli cells exposed to MODERATE SOUND

The cellular morphology of E. coli was analyzed 48 hours after sound exposure. Using SEM software, the length and width of an E. coli cell were measured. The average length of E. coli was determined to be 2.060 ± 0.485 μm (at 80 dB) and 2.395 ± 0.904 μm (at 100 dB), respectively. Under a sound intensity level of 100 dB, the length increased by more than 27.26% compared to the control group (1.882 ± 0.375 μm). However, there was no discernible difference in width.

D. Effects of sound on ESBL-E. Coli antibiotics susceptibility

Ciprofloxacin, trimethoprim/sulfonamides, gentamicin, and amoxicillin/clavulanic acid, previously resistant antibiotics, all exhibited sensitivity in the test following treatment. The response of ESBL-producing E. coli (ESBL-E. coli) to auditory sound stimulation from the sound wave, especially concerning multi-drug-resistant ESBL-E. coli, underscores the significance of moderate sound. however, no improvement was observed in the effectiveness of the moderate sound when probiotic bacteria from Kefir and kombucha were introduced to a single sample. Before treatment, ESBL-E. coli was susceptible to Clavulanic Acid but resistant to all other

antibiotics. If an ESBL-E. coli test utilizing Kamboucha + Kefir is employed (refer to Table 2).

4. Discussion

Public health primarily focuses on protecting people's health, including limiting healthcare inequities and improving healthcare quality and accessibility. According to the Centers for Disease Control and Prevention (CDC) [11], there are thirty-one recognized foodborne pathogens, including E. coli, and unidentified agents that can infect humans. Sound, a mechanical wave, is produced by particles in a medium vibrating back and forth. When sound waves travel through living organisms, they can have biological effects, such as displacing cells. Certain organisms may exhibit beneficial growth responses to auditory stimuli [9]. Different sound exposures, varying in amplitude, frequency, and duration, can cause interspecies differences in growth, biomass, and intracellular chemical production, significantly impacting various ecological processes [12].

This study investigated how E. coli cells react to auditory sound stimulation under both relaxed and stressful conditions. The results showed that colony formation in E. coli increased significantly under typical growth conditions when exposed to audible sound. Specifically, sound stimulation enhanced E. coli growth, with growth promotion increasing as the sound frequency increased. Exposure to sound frequencies of 2 kHz and 8 kHz resulted in significant differences ($P<0.001$) in biomass, which increased by approximately 21.04% and 27.06%, respectively, due to an increase in the μmax, indicating faster growth. These findings align with those of Shao-Bin Gu et al.; 2013 [13], who found that audible sound waves stimulated E. coli growth. When the vibration frequency matches the microbial cells' natural frequency, they can absorb more energy [14].

Sound stimulation at specific strengths may promote the development of E. coli, as shown in studies on sound waves at varying intensities and power levels. Our research found that a high sound power level (between 55 and 63 dB) with a consistent sound frequency of 8 kHz and an intensity level of 80 dB reduced the impacts of E. coli growth promotion. Similar findings were reported by Gu et al.; 2016 [9] biological macromolecules like proteins, lipids, nucleic acids, and polysaccharides must rapidly accumulate inside cells for cell division [15]. Early in treatment, sound exposure significantly increased intracellular protein production. The treated group's intracellular protein value at 6 hours was 566.4 mg/g, 1.1 times higher than the control group. This result is consistent with Yang's findings that sound stimulation can promote the synthesis of intracellular molecules such as protein [16].

Sound stimulation can also induce changes in cell structure, affecting growth, metabolism, and division [9]. In this investigation, the average length of E. coli cells increased by more than 29.67%. The stimulation group experienced maximum biomass and a specific growth rate about 1.8 and 3.4 times higher than the control group when exposed to a 7900 Hz, 90 dB sound wave. The responses of bacterial cells to sound stress may involve several pathways. The results are consistent with Vadia and Levin [17], who found that cell size is a linear function of growth rate. High-intensity sound waves can destroy microorganisms by breaking down their cell walls, while low-intensity sound waves may increase cellular metabolism, enhancing growth and reproduction [18]. This phenomenon, known as sonoporation, has been researched as a potential treatment for bacterial infections.

Antibiotic resistance continues to accelerate due to various human actions. This study's findings can significantly contribute to our understanding of the many processes of antibiotic resistance, opening new avenues for developing effective antibiotic therapies [19]. Four antibiotics—Trimethoprim/Sulfamethoxazole, Ciprofloxacin, Amoxicillin/Clavulanic Acid, and Gentamicin—showed resistance to ESBL-E. coli and were selected for testing after sound trearing based on this investigation's results. Following treatment, susceptibility tests showed positive results for ciprofloxacin, trimethoprim/sulfonamides, gentamicin, and amoxicillin/clavulanic acid. ESBL-E. coli responded better to the auditory sound stimulation. The results of sound test on multidrug-resistant ESBL-E. coli are noteworthy. No improvement was observed when probiotic bacteria from Kefir and Kombucha were introduced to a sample to enhance the sound. Before treatment, the findings showed resistance to all antibiotics except amoxicillin, to which clavulanic acid is responsive. When testing for ESBL-E. coli using the Kombucha + Kefir test, Quranic verses, particularly those more potent than others, profoundly affected the E. coli bacteria.

5. Conclusion

The sound of specific moderate sound can be used to create sound clips that target bacteria, potentially eliminating or reactivating them. This investigation provides evidence that the sound frequency invention device operates as intended. This technology could transform antibiotic-resistant germs into susceptible ones. Additionally, sound treatment has been shown to reduce biofilm production, which is crucial for the energy needs of microorganisms for growth and reproduction. The effectiveness of sound treatment depends on various factors, including the type of microbe being treated and the duration and intensity of the exposure.

Conflict of interest

Author declares no conflict of interests. Author reads and approved final version of the paper.

References

[1] Sader, H. S., Mendes, R. E., Streit, J. M., Carvalhaes, C. G., & Castanheira, M. (2022). Antimicrobial susceptibility of Gram-negative bacteria from intensive care unit and non-intensive care unit patients from United States hospitals (2018–2020). *Diagnostic Microbiology and Infectious Disease, 102*(1), 115557.

[2] Collignon, P., MacKinnon, M. C., McEwen, S. A., Pearl, D. L., Lyytikainen, O., Jacobsson, G., ... & Laupland, K. B. (2021). Mortality in

Sample No.	Organism	Antibiotic	Before treatment	After treatment		
			Resistant	moderate sound	Kombucha with moderate sound	KEFIR with moderate sound
1	ESBLE. coli	Ciprofloxacin	R	S	-	-
2	ESBLE. coli	Ciprofloxacin	R	R	-	-
		Trimethoprim/Sulfa	R	R		
3	ESBLE. coli	Ciprofloxacin	R	R	-	-
		Trimethoprim/Sulfa	R	R		
4	ESBLE. coli	Ciprofloxacin	R	R	-	-
		Trimethoprim/Sulfa	R	R		
8	ESBLE. coli	Ciprofloxacin	R	S		
		Trimethoprim/Sulfa	R	R		
9	ESBLE. coli	Trimethoprim/Sulfa	R	R	-	-
13	ESBLE. coli	Ciprofloxacin	R	R	-	-
		Trimethoprim/Sulfa	R	R	-	-
14	ESBLE. coli	Ciprofloxacin	R	R	-	-
		Amoxicillin/Clavulanic Acid	R	R	-	-
15	ESBLE. coli	Ciprofloxacin	R	S	-	-
		Trimethoprim/Sulfa	R	S	-	-
16	ESBLE. coli	Ciprofloxacin	R	R	-	-
		Trimethoprim/Sulfa	R	R	-	-
18	ESBLE. coli	Ciprofloxacin	R	-	-	-
		Trimethoprim/Sulfa	R	-	-	-
19	ESBLE. coli	Ciprofloxacin	R	-	-	-
		Trimethoprim/Sulfa	R	-	-	-
21	ESBLE. coli	Ciprofloxacin	R	-	-	-
22	ESBLE. coli	Ciprofloxain	R	-	-	-
		Trimethoprim/Sulfa	R	-		
23	ESBL E. coli	Gentamicin	R	-		
24	ESBL E. coli	Ciprofloxacin	R	-		
25	ESBLE. coli	Ciprofloxacin	R	-		
27	ESBL E. coli	Ciprofloxacin	S	-	-	-
29	ESBLE. coli	Ciprofloxacin	R	-	-	-
		Trimethoprim/Sulfa	R	-		
30	ESBL E. coli	Ciprofloxacin	R	-	-	-
		Trimethoprim/Sulfa	R	-		
31	ESBL E. coli	Ciprofloxacin	R	-		
		Trimethoprim/Sulfa	R	-		
33	ESBLE. coli	Trimethoprim/Sulfa	R	-		
35	ESBL E. coli	Ciprofloxacin	R	-	R	R
		Trimethoprim/Sulfa	R	-	R	R
36	ESBLE. coli	Amoxicillin/Clavulanic Acid	S	-	-	-
		Trimethoprim/Sulfa	S	-		
41	ESBLE. coli	Trimethoprim/Sulfa	R	S*		
43	ESBLE. coli	Ciprofloxacin (CONTROL)	S	S	-	-
43		Trimethoprim/Sulfa (CONTROL)	S	S#		
44	ESBLE. coli	Trimethoprim/Sulfa	R	R*		
46	ESBLE. coli	Ciprofloxacin	R	R	-	-
		Trimethoprim/Sulfa	R	S		
47	ESBLE. coli	Ciprofloxacin	R	R	-	-
		Trimethoprim/Sulfa	R	R*		

Table 2: The effect of frequency of against the ESBL-E. Coli bacteria, which were previously resistant to antibiotics

Escherichia coli bloodstream infections: a multinational population-based cohort study.

[3] Gopaul, S., Dominic, C., Tinhuna, J., Green, J., Watkins, E., & Melzer, M. (2023). Hospital-acquired E. coli bacteraemia at a large UK NHS Trust. A return towards baseline following implementation of a 5-year quality improvement programme. *Infection Prevention in Practice, 5*(2), 100280.

[4] Shakya, P., Shrestha, D., Maharjan, E., Sharma, V. K., & Paudyal, R. (2017). ESBL production among E. coli and Klebsiella spp. causing urinary tract infection: a hospital based study. *The Open Microbiology Journal, 11*, 23.

[5] Abalkhail, A., AlYami, A. S., Alrashedi, S. F., Almushayqih, K. M., Alslamah, T., Alsalamah, Y. A., & Elbehiry, A. (2022, September). The prevalence of multidrug-resistant Escherichia coli producing ESBL among male and female patients with urinary tract infections in Riyadh Region, Saudi Arabia. In *Healthcare* (Vol. 10, No. 9, p. 1778). MDPI.

[6] Saipriya, J. B., Shubha, D. S., Sudhindra, K. S., Sumantha, A., & Madhuri, K. R. (2018). Clinical importance of emerging ESKAPE pathogens and antimicrobial susceptibility profile from a tertiary care centre. *Int J Curr Microbiol Appl Sci, 7*(5), 2881-2891.

[7] Harris, A., Lindsay, M. A., Ganley, A. R., Jeffs, A., & Villas-Boas, S. G. (2021). Sound stimulation can affect Saccharomyces cerevisiae growth and production of volatile metabolites in liquid medium. *Metabolites, 11*(9), 605.

[8] Sarvaiya, N., & Kothari, V. (2017). Audible sound in form of music can influence microbial growth, metabolism and antibiotic susceptibility. *J. Appl. Biotechnol. Bioeng, 2*(6), 00048.

[9] Gu, S., Zhang, Y., & Wu, Y. (2016). Effects of sound exposure on the growth and intracellular macromolecular synthesis of E. coli k-12. *PeerJ, 4,* e1920.

[10] Ku, H. N., Lin, W. F., Peng, H. L., & Yew, T. R. (2021). In-situ monitoring the effect of acoustic vibration in the form of music on the motility of Escherichia coli. *Applied Acoustics, 172,* 107620.

[11] Feng, L., Song, B., Zang, Z. F., Sun, N. L., Wang, J. S., Liu, F., & Lei, S. W. (2018). Discussion on implementation of public health standards adopted in centers for disease control and prevention. *Zhonghua liu Xing Bing xue za zhi= Zhonghua Liuxingbingxue Zazhi, 39*(9), 1287-1290.

[12] Robinson, J. M., Cameron, R., & Parker, B. (2021). The effects of anthropogenic sound and artificial light exposure on microbiomes: ecological and public health implications. *Frontiers in Ecology and Evolution, 9*, 662588.

[13] Gu, S. B., Yang, B., Wu, Y., Li, S. C., Liu, W., Duan, X. F., & Li, M. W. (2013). Growth and physiological characteristics of E. coli in response to the exposure of sound field. *Pakistan journal of biological sciences: PJBS, 16*(18), 969-975.

[14] Reguera, G. (2011). When microbial conversations get physical. *Trends in microbiology, 19*(3), 105-113.

[15] Pérez Castro, S., Borton, M. A., Regan, K., Hrabe de Angelis, I., Wrighton, K. C., Teske, A. P., ... & Ruff, S. E. (2021). Degradation of biological macromolecules supports uncultured microbial populations in Guaymas Basin hydrothermal sediments. *The ISME Journal, 15*(12), 3480-3497.

[16] Yang B. Thesis. A research on the effects of audible wave exposure on

the physiological and ecological characteristics of Escherichia coli and its mechanism. 2013.

[17] Vadia, S., & Levin, P. A. (2015). Growth rate and cell size: a re-examination of the growth law. *Current Opinion in Microbiology, 24*, 96-103.

[18] Tomizawa, M., Shinozaki, F., Motoyoshi, Y., Sugiyama, T., Yamamoto, S., & Sueishi, M. (2013). Sonoporation: Gene transfer using ultrasound. *World Journal of Methodology, 3*(4), 39.

[19] Chinemerem Nwobodo, D., Ugwu, M. C., Oliseloke Anie, C., Al-Ouqaili, M. T., Chinedu Ikem, J., Victor Chigozie, U., & Saki, M. (2022). Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. *Journal of Clinical Laboratory Analysis, 36*(9), e24655.