Trop J Nat Prod Res, May 2021; 5(5):825-830 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Study of the Effects of Audible Sounds and Magnetic Fields on *Staphylococcus aureus* Methicillin Resistance and *mecA* Gene Expression

Mustafa A-M. Ali and Bahaa A. L. Al-Rubaii*

University of Baghdad, College of Science, Department of Biology.Baghdad, Iraq

ARTICLE INFO

Article history:
Received 03 January 2021
Revised 18 March 2021
Accepted 19 May 2021
Published online 03 June 2021

ABSTRACT

Methicillin Resistant *Staphylococcus aureus* (MRSA) is one of the most dangerous antibiotic resistant strains due to the wide range of illnesses it can cause. For this reason, the study aimed to evaluate the effect of different physical parameters (audible sound and magnetic fields) on resistance pattern and *mec*A gene expression of MRSA strains. A total of 193 *S. aureus* clinical isolates were collected from local hospitals in Baghdad, Iraq. Antibiotic disc diffusion method was employed to identify MRSA using cefoxitin antibiotic, and to examine the resistance of the isolates to gentamycin, tetracycline, penicillin G and ciprofloxacin. Effect of two audio treatments; heavy metal music and classic music and two magnets (65 mT/600 Gauss and 80 mT/800 Gauss) were investigated on methicillin resistance of multidrug resistant strains MRSA and their *mec*A gene expression. MRSA comprised 27.9%, while antibiotic resistance profiling revealed that 5.5% were multidrug resistant MRSA. Furthermore, when exposed to heavy metal music, isolates showed a small increase in sensitivity diameter, while on exposure to classical music, isolates showed no increased sensitivity. The inhibitory zone diameter was enlarged, and the susceptibility of all treated isolates appeared to be sensitive. Magnetic fields treatments, on the other hand, also showed increased susceptibility of isolates compared to control, with the large magnet having a potent effect in terms of making the tested isolates completely sensitive. The expression of *mecA* gene under the effect of classic music and large magnet was down-regulated by 0.07- and 0.03-fold changes, respectively, using Real-Time qPCR.

Keywords: *S. aureus*, MRSA, Heavy metal music, Classic music, Magnetic fields.

Introduction

Staphylococcus aureus is a prominent and well-known global pathogen that causes diverse clinical infections, alternating from minor superficial infections, such as boils, to systemic and life threating infections like bacteremia. It also causes food poisoning; device related infections (catheters), wound infections as well as urinary tract infection.[1] This bacterium is also a commensal of the skin and nasopharynx cavity.[1] Methicillin Resistant *Staphylococcus aureus* (MRSA) was initially isolated during the sixties. MRSA infections were first recorded in hospital settings and were denoted as "hospital-acquired MRSA (HA-MRSA)". Yet, in the late nineties, community-acquired MRSA (CA-MRSA) infections started to appear in healthy individuals with no recognized risk-factors for such highly resistant strains-infections.[2] MRSA now respond only to very advanced antibiotics that were never meant to be a first-line defense due to antibiotic misuse and abuse.[3] One of the approaches in the medical field is the use of physical means to reduce resistance, as Ayan *et al.* have shown in their 2008 study that low intensity pulsed sound can affect *S. aureus* on a morphological and molecular level.[4] A study in 2010 stated that sound in the form of music affects aerobic bacteria substrate utilization from wastewater of treatment plant.[5] Researchers have also found that audio therapy is successful in reducing resistance, influence growth and affect metabolites and toxin production in various bacterial species.[6,7] Others, like Kamel *et al*., demonstrated in a local study in 2014 that exposing *S. aureus* to high frequency magnetic fields for a short period of time affects the resistance pattern of the microbe and change some of its biochemical properties.[8] Also Brkovic *et al.* demonstrated in their study conducted in 2015 that magnetic fields indeed have a positive effect by reducing the number of microbes isolated from dental plagues using modified optical density (OD) of agar turbidimetry assay, microbial count and spectrophotometry[9]. Thus, our study aimed to evaluate two different physical parameters (audible sounds and magnetic fields) on resistance pattern of MRSA to cefoxitin and *mec*A gene expression of multidrug resistant MRSA.

*Corresponding author. E mail: bahaa.abdullah@sc.uobaghdad.edu.iq
Tel: +9647709780888

Citation: Ali MAM and Al-Rubaii BAL. Study of the Effects of Audible Sounds and Magnetic Fields on *Staphylococcus aureus* Methicillin Resistance and *mecA* Gene Expression. Trop J Nat Prod Res. 2021; 5(5):825-830. doi.org/10.26538/tjnpr/v5i5.6

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Materials and Methods

Isolation and identification of bacterial strains

Clinical isolates included a total of one hundred and ninety-three S. *aureus* isolates, collected from local hospitals (Alameerat hospital private, Madynat Al-tib Hospital and Janeen Private Hospital) in Baghdad, Iraq. These isolates comprised of urine specimens, wound infection swabs and burn infection swabs (88), (63) and (44) respectively. Transport media (Biosigma, Italy) were inoculated with the burn and wound samples swabs followed by culture activation on brain-heart infusion broth (Hi media, India) and purified with sub-culturing on Mannitol-Salt agar (Hi media, India), while urine sample isolates were streaked directly onto blood agar and mannitol-salt agar for primary identification.

Molecular identification of S. aureus

Molecular diagnosis was employed using Polymerase Chain Reaction (PCR) technique to further confirm phenotypic identification of *S. aureus*. This was done by amplifying the 16S rRNA gene of *S. aureus* using the following primers, F**:** 16S rRNA 5'-GTAGGTGGCAAGCGTTACC-3' and R: 5'-CGCACATCAGCGTCAG-3', with a product size of 288

bp.[10] DNA was extracted from overnight bacterial suspension using DNA extraction kit (AddBio, South Korea), followed by measuring the concentration of DNA using Qubit 4.0 instrument (Invitrogen, USA), amplification using PCR and visualization on agarose gel (2.5%) after separation by electrophoresis technique (Figure 2). Identification of MRSA was also confirmed using conventional PCR utilizing the following primers, F: *mec*A 5'-TCCACCCTCAAACAGGTGAA-3' and R: 5'-TGGAACTTGTTGAGCAGAGGT-3', producing an amplicon size of 139 (Figure 2).[13]

Antibiotic disc diffusion test
The Kirby-Bauer disc diffusion method was adopted to study the antibiotic resistance of tested isolates.[11] Methicillin resistance isolates were identified using cefoxitin (Mast, Germany) as alternative to methicillin due to its high sensitivity to detect *mecA* gene. Overnight isolates were cultured on brain-heart infusion broth, then turbidity was adjusted to McFarland tube number 0.5, swabbed uniformly on Muller-Hinton agar and the discs were dispersed on the surface of the agar using a sterile forceps and incubated for 24 hrs at 37^{o}C. Multi-drug resistant MRSA isolates were screened using the same procedure with four different antibiotics (penicillin G, gentamycin, tetracycline and ciprofloxacin).

Treating MRSA with Audible Sound
Overnight suspension of each multidrug resistant MRSA isolate was adjusted to match the turbidity of McFarland tube number 0.5 for standardization and inoculation into 1 ml appendroff tubes and placed inside a locally assembled sound amplifying device (Slipknot box). The device was placed inside the incubator and the samples were subjected to desired sound pattern via mobile phone-AUX connection (Heavy metal music with mean frequency: 612 Hz, mean Loudness: 81 Decibels and classic music with mean frequency: 501 Hz, mean loudness: 71 Decibels) in separate experiments. Triplicates for each isolate and control were made from inoculated tubes inside the audio device without any audio input. Methicillin/cefoxitin antibiotic susceptibility was checked after treatments to monitor phenotypic resistance patterns changes by Kirby-Bauer methods.[11]

Treating MRSA with magnetic fields
Overnight activated isolates in Brain-Heart infusion broth were adjusted to match the turbidity of McFarland tube number 0.5 and inoculated into new plain tubes. Each isolate was made into three replicates. Magnetic fields were exerted onto the tubes using conventional magnets (Small magnet 65 mT/ 600 Gauss and large magnet 80 mT/ 800 Gauss). The magnets were incubated with the tubes overnight and an iron plate was placed beneath the tubes to ensure a uniform magnetic field flow. To screen for any changes in phenotypic resistance, Methicillin/Cefoxitin disc diffusion method was employed after incubation. Control group was made by incubating inoculated tubes without magnets.

Gene expression
The gene expression of *mec*A gene was monitored using Quantitative Real-Time PCR assay to evaluate the potency of treatments under study on the resistance pattern of MRSA, while the 16S rRNA gene served as housekeeping. Triplicates of each isolate were incubated overnight and cells were harvested to extract RNA using RNA extraction kits (Geneaid, South Korea) following the manufacturers' protocol for RNA extraction. The concentration of the extracted and purified RNA was measured using Qubit 4.0 instrument (Invitrogen, USA) following the manufacturer's instructions. This was followed by cDNA synthesis using Protoscript kit (NED, UK) as follows: 5 µl from each extracted RNA of every sample added into new separated PCR tube and 10 µl Protoscript reaction mix (contains dNTPs, buffer and other essential components for the reaction), 2 µl of reverse transcriptase enzyme and 2 µl of unique primers were added, followed by 1 µl of free-nuclease water to bring the total volume to 20 µl, this mixture was incubated for 1 hour at 42 ºC using a thermo-cycler followed by elevation of temperature to 80 ºC for inactivation of enzyme. The cDNA product was stored until used for qPCR procedure. 12.5 µl of Luna Universal qPCR Luna universal Master Mix (NED / UK) was added to the qPCR setup, and added 1.5 µl forward primer (10 µM), 1.5 µl reverse primer (10 µM), 4 µl template RNA (4.41 to 60.7 ng/µL) and 0.5 µl Nuclease-free Water to complete a 20 µL reaction volume. The detection of mRNA quantity was based on fluorescent power of SYBRGreen and the thermo-cycling protocol program was setup as follows: Initial denaturation at 95 °C for 60 seconds for one cycle, denaturation at 95 °C for 15 seconds, extension at 60 °C for 30 seconds for 40 cycles and finally melt curve at 60-95 °C for 90 minutes for one cycle, and the results were collected and analyzed by using the following formula below as described by Livak and Schmittgen[14]:

$$\Delta Ct = Ct_{target} - Ct_{Housekeeping} : \Delta\Delta\ Ct = \Delta Ct_{Treated} - \Delta Ct_{Control} : folding = 2^{-(\Delta\Delta Ct)}$$

The primers used for gene expression are listed in Table 1.

Statistical analyses
All the data were presented as mean of at least three replicates. One-way ANOVA, Chi square and T-test with $\alpha = 0.05$ were used to calculate the significance.

Results and Discussion

Identification of S. aureus and MRSA
All one hundred and ninety-three clinical *S. aureus* isolates were initially identified by: gram staining and the isolates exhibited the typical tetrad configuration, 100 % of the isolates were mannitol fermenters, 100 % catalase positive, 100 % oxidase negative. Furthermore, 76 % were coagulase positive, 100 % were MR-VP positive and finally 85 % displayed beta hemolysis. Further identification was based on molecular techniques by amplifying the 16S rRNA gene of *S. aureus* using conventional PCR and visualizing the results on 2.5% agarose with amplicon size of 228 bp, confirming the isolates are indeed *S. aureus* as shown in Figure 2. Furthermore, based on initial identification of MRSA using cefoxitin disc diffusion test, 54 (27.9 %) of the isolates were methicillin/cefoxitin resistant (Figure 1). The results were interpreted based on CLSI (2018)[12] data as diameters of inhibition zones in millimeters (≤ 21 are considered resistant while > 21 are considered sensitive). Confirmation of MRSA was also screened using conventional PCR by amplifying *mec*A gene. The fractionation revealed a 139 bp band when visualized on 2.5 % agarose as depicted in Figure. 2.
Al-Ugaili's study in 2013 on clinical isolates taken from different hospitals in Baghdad revealed that 29.6 % of samples were confirmed to be *Staphylococcus aureus*,[15] while, Younan, *et al*[17] obtained the highest *S. aureus* samples from burn swab (35 %), followed by urine specimen (30 %). Furthermore, a regional study conducted in Iran revealed that, *S. aureus* formed nearly 30.8 % from urine specimens, 26.5 % of wound swabs and 22.7 % of burn swabs.[18] Variation in numbers among different studies is only logical due to variation in clinical settings in different hospitals, differences in clinical isolation sites, population dissimilarity and infection treatments protocols variation by the patients themselves or by healthcare institutes. Al-Dahbi and Al-Mathkhury reported a local study in Baghdad that RT-q PCR was used to screen for *mecA* gene in *S. aureus* isolates and their results were in accordance with that obtained when cefoxitin disc diffusion test was used for investigating MRSA. Moreover, the researchers stated that 94.3 % of isolates were MRSA.[19] Another local study demonstrated that, 100 % of clinical isolates of *S. aureus* were MRSA as *mecA* gene was identified by PCR.[16] Another study in Iran found that cefoxitin has a high specificity for the screening of MRSA, with 46.3 % of isolates being Methicillin-Resistant *Staphylococcus aureus*. [21] One study performed in Saudia Arabia analyzed a total of 830 individuals and a total of 164 (19.8 %) were found to be colonized by *S. aureus*, and of these, 38 (4.6 %) were MRSA. Thus, the MRSA rate amongst all *S. aureus* carriers was 23.2 %.[22]

MRSA antibiotic profiling
All the 54 MRSA isolates were profiled for their resistance to several other generic antibiotics commonly used by the population for

bacterial infections. The results were variable in terms of resistance, as 41 (75.9 %) were resistant to penicillin G, 14 (25.9 %) were resistant to gentamycin and 6 (11.1%) were intermediate, 5 (9.2%) were resistant to ciprofloxacin and 2 (3.7%) were intermediate, finally 12 (22.2%) were resistant to tetracycline and 4 (7.4%) were intermediate, as illustrated in Figure 3.

The results also revealed that 3 (5.5%) were multidrug resistant to tested antibiotics**.** A study conducted by Al-Dahbi and Al-Mathkhury (2013) to profile MRSA isolated from local hospital healthcare workers found that 100% of the tested isolates were resistant to penicillin G, 34.9% were resistant to tetracycline, 29.3% were resistant to gentamicin, and 29.2% were resistant to ciprofloxacin.[19] Another local study conducted by Abd Al-Redha in 2018 showed a similar percentage of MRSA resistance to ciprofloxacin (8%), an observation similar to our study.[16] However, Dibah *et al.* profiling of MRSA showed that 100% of hospital isolates were resistant to Penicillin G, 84.2% were resistant to Tetracycline, 68.4% were resistant to Ciprofloxacin and 78.9 % were resistant to Gentamycin.[21] Variation in resistance to different antibiotic is not a coincidence as it is the result of geographical differences, the difference of clinical in isolation sources, infection therapy protocols and drug abuse by patients.

Audio therapy

Our study evaluated the effect of audible sound in the form of heavy metal music and classic music on three multidrug resistant MRSA strains using the locally assembled Slipknot box. The phenotypic results revealed that heavy metal audio treatment affected the resistance pattern of all three isolates under study and increased their sensitivity to cefoxitin compared to control (16 to 20 mm in diameter), interesting finding of one isolate shifted towards being completely sensitive (more than 21 mm). Classical music treatment, on the other hand, affected all three treated isolates, as all of them shifted towards being sensitive (above 22 mm in diameter according to CLSI[12]) to cefoxitin compared to resistant controls as demonstrated in Figure 4.

Sarvaiya and Kothari study in 2017 on the influence of sound waves in the form of Indian classic music (Raag Malhar), with a frequency range of 41-645 Hz, on the growth, antibiotic susceptibility and metabolism of many microorganisms revealed that *S. aureus* susceptibility to antibiotics increased by 35.66 % under the influence of the sound treatment.[23] Other researchers reported the effect of sound in different frequencies on other *S. aureus* parameters such as growth and metabolism. For example, Kotwal *et al*. study in 2016 investigated different sound patterns in the form of different music types (Heavy metal, classic Mozart music, Bollywood music and Gayatri mantra) on the growth of *S. aureus*. The results showed an increase in growth pattern of *S. aureus* under all treatments compared to control with heavy metal music showing the highest result.[25] It is not clear how the sound affects the bacterial growth, metabolism or antibiotic susceptibility. However, some researchers pointed that membrane permeability of the tested gram-positive microorganism increased in response to sound stimulation (172-581 Hz), affecting glucose utilization by *Brevibacillus parabrevis*.[6] The decrease in resistance of *S. aureus* might be attributed to the mechanical effect of sound waves on the expression of resistance gene. This can be either during transcription such as the DNA polymerase failing to attach and dissociate properly from DNA template or during translation such as during protein configuration and bonds formation. We also think that the mechanical force of sound waves may affect the ion exchange channels of cell membrane that might cause a disturbance in the biology of microbial cell and thus affecting its susceptibility to cefoxitin as the antibiotic was able to penetrate the cell and inhibit its growth.

Magnetic fields effect

The results were exciting as resistance under small magnet (600 Gauss/65 mT) was decreased and all tested isolates were sensitive to cefoxitin. On the other hand, the large magnet (800 Gauss/80 mT) had even more potent effect decreasing the resistance of tested isolates, as they became increasingly sensitive to cefoxitin compared to control as depicted in Figure 5.

Small number of published literature considering the effect of magnetic fields on antibiotic susceptibility of *S. aureus* was found. However, a local study conducted by Kamel *et al.* on growth and antibiotic susceptibility of S. *aureus* under the effect of magnetic forces of (400, 800, 1200, 1600 Gauss). The study concluded that the growth of *S. aureus* was increased on the first few hours of treatment compared to control and decreased gradually for 20 hours after.[8] Another study on gram-positive *Bacillus* sp. showed that the sensitivity of the tested bacterium to trimethoprim and ampicillin drugs increased when treated with a magnetic field of 0.2 mT and 0.3 mT for 10 minutes.[27] The mechanism by which the magnetic fields affect bacterial growth or antibiotic resistance is not fully understood as all living entities were in continuous exposure to magnetic fields on daily basis. It is possible that magnetic field affected the surface charges of the membrane and thus affecting its permeability to ions and/or antibiotics molecules. Furthermore, it might affect the charge distribution on the antibiotic molecule leading to the modification of the rate of penetration of cell by the antibiotic, causing the increased susceptibility to antibiotics. It might as well have affected the protein configuration during the processing and folding of protein to its final functional form, leading to disturbance in the functionality of the cell biology and possible effect on cell wall synthesis, leading to decreased antibiotic resistance.

Gene expression of mecA gene

According to our phenotypic study results, two of the three treated isolates were checked for their gene expression activities after treatments using quantitative Real-Time PCR. The two most affected isolates of our under-study treatments were selected to undergo gene expression. Resistance to cefoxitin was phenotypically reduced remarkably under the effect of classic music audio treatment and large magnet treatment, thus those were the treatments used in quantitative PCR to monitor if there was any variability in their gene expression. The gene that was studied for its expression was *mecA*, the main gene responsible for methicillin/cefoxitin resistance, while the 16S rRNA served as a house-keeping gene. Variable results were observed in the gene expression, some of them matched the phenotypic results as the gene expression of *mecA* was reduced, others, however, induced the expression of the gene as shown in Table 2.

For classic music, one isolate, down-regulation was observed for all genes. The other isolate, however, showed an up-regulation in the expression of all tested genes. Additionally, for magnetic treatment, a down-regulation was observed in three occasions for one isolate of all the genes. On the other hand, the other isolate also showed an up-regulation of all the genes. Potenza *et al.* publication in 2004 demonstrated that magnetic fields caused variation in gene expression due to DNA damage by free radicals or by inducing point mutations in genes which lead to up-regulation or down-regulation of those genes.[28] Fijałkowski *et al.* reported in 2016 that gene expression of *S. aureus* enterotoxins was down-regulated when exposed to magnetic fields of 34 mT. They also shown that the treatment affected the growth rate of the microbe.[29,30] Our point of view regarding the variation in gene expression of the genes under study is mainly due to strain variation, as different strains of *S. aureus* might react differently to the treatments under study and thus led to the variations. On the other hand, the effect of each treatment on the up or down regulation of the target genes is unclear, as no study has explored such type of treatments on those genes. However, those point mutations are the most likely cause of the variance, as different strains produced varied expression of the genes. Experiment technical error and primers' specificity might have contributed to the variations. Mechanical effects of treatments might have affected the gene expression by mechanical disruption of the proteins produced during cell division, as the bacterial population was exposed to the treatments for 24 hrs. Finally, due to the net charge of ions in the cytoplasm, the net charge produced by magnetic field may have an effect on gene expression, influencing the activity of DNA or RNA polymerase, and consequently the transcription/translation process as a whole.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: List of all primers used for *S. aureus* identification in conventional PCR and for gene expression in qPCR

Primer	Primer Sequence 5′→3′	Target	Fragment size (bp)	Reference
***mecA* F**	TCCACCCTCAAACAGGTGAA	*mec*A	139	13
***mecA* R**	TGGAACTTGTTGAGCAGAGGT			
16S rRNA F	GTAGGTGGCAAGCGTTACC	16S rRNA	288	10
16S rRNA R	CGCACATCAGCGTCAG			

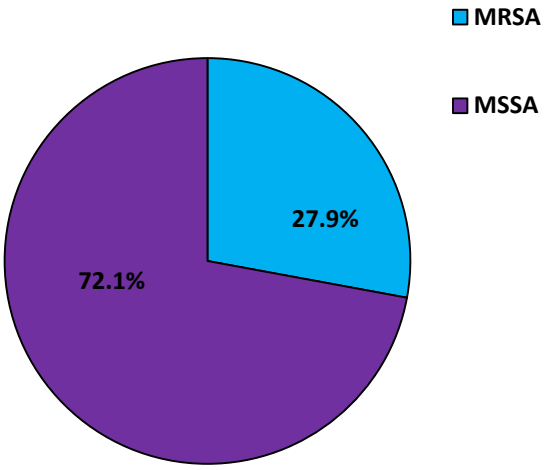

Figure 1: Percentage of MRSA (**MRSA:** Methicillin Resistant *Staphylococcus aureus*; **MSSA:** Methicillin Sensitive *Staphylococcus aureus*).

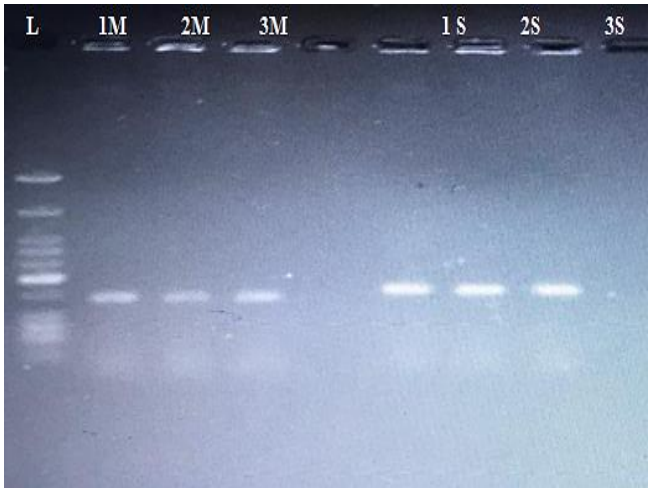

Figure 2: Screening of *Staphylococcus aureus mecA* (M) and 16S rRNA (S) genes on 2.5% agarose gel. Lane L: 100 bp ladder. Lanes 1-3: 139 bp of *mec*A; lanes 5-7: 228 bp of 16S rRNA.

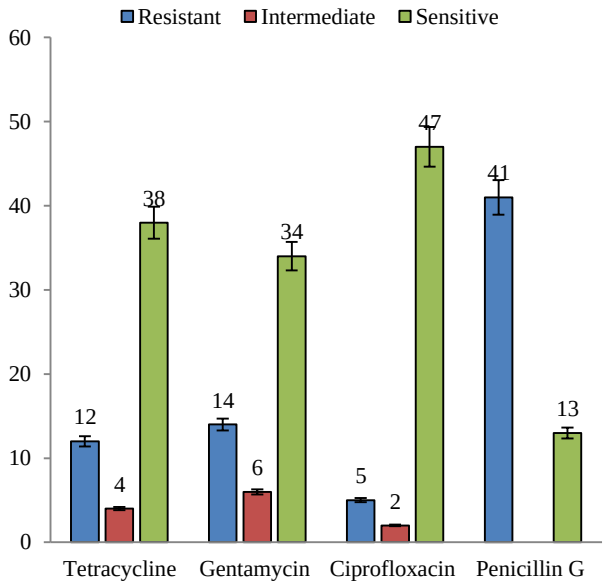

Figure 3: MRSA numbers for antibiotics susceptibility profiling

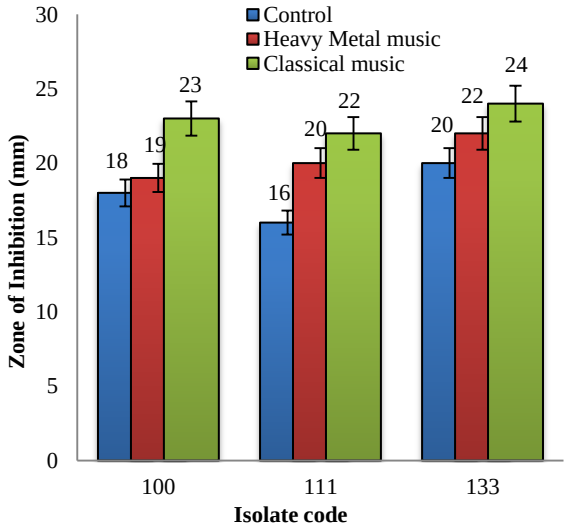

Figure 4: Effect of audible sound on the resistance pattern of MRSA (diameter in mm)

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

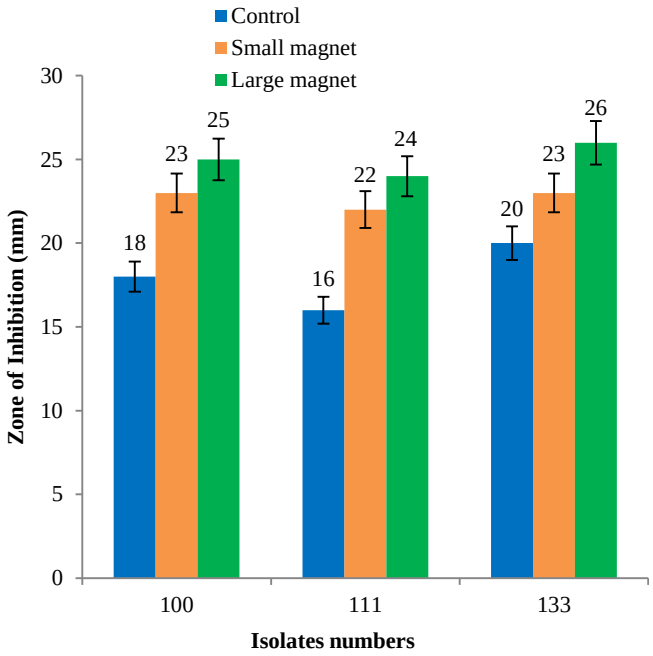

Figure 5: Magnetic effect on MRSA antibiotic resistance (diameter in mm)

Table 2: The effect of different treatments on *mec*A gene expression fold change

Isolate number	Classic music	Large magnet
100	0.07	0.03
111	4.9	12.5

*****control is 1. **Below 1 is down-regulation. ***Above 1 is up-regulation

Conclusion

From the results presented, we concluded that physical forces in form of audio or magnetic fields affected the resistance pattern of MRSA against cefoxitin. This makes the tested isolates sensitive and susceptible to antibiotic therapy. They also might have a direct or indirect effect on the gene expression of the *mec*A gene, making the mentioned treatments promising for future applications and research.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors would like to express their gratitude Mustapha Kamel, Obaida Ali and Maytham Abdulkareem Yasir Samir and Zaidoon Monaam for their help to complete this study.

References

1. Tong S, Davis J, Eichenberger E, Holland T, Fowler V. *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015; 10(5):855-857.
2. Sharma S, Srivastava P, Kulshrestha A, Abbas A. Evaluation of different phenotypic methods for the detection of methicillin resistant *Staphylococcus aureus* and antimicrobial susceptibility pattern of MRSA. Int J Comm Med Pub Heal. 2017; 4(9):3297-3301.
3. Walsh T and Howe R. The Prevalence and mechanisms of vancomycin resistance in *Staphylococcus aureus.* Annu Rev Microbiol. 2002; 56:657-75.
4. Ayan I, Aslan G, Cömelekoğlu U, Yilmaz N, Colak M. The effect of low intensity pulsed sound waves delivered by the exogen device on *Staphylococcus aureus* morphology and genetics. Acta Orthop Traumatol Turc. 2008; 42(4):272-277.
5. Pornpongmetta S and Thanuttamavong M. Effects of music on microbial substrate utilization of aerobic bacteria from municipal wastewater treatment plant part II: comparative effects of musical characteristics. J Res Eng Tech. 2010; 7(2):41-48.
6. Shah A, Raval A, Kothari V. Sound stimulation can influence microbial growth and production of certain key metabolites. J Microbiol Biotech Food Sci. 2016; 5(4):330-334.
7. Banerjee S, Goswami A, Datta A, Pyne A, Nikhat, A, Ghosh, B. Effect of different sound frequencies on the growth and antibiotic susceptibility of *Escherichia coli*. Int J Curr Microbiol Appl Sci. 2018; 7(3):1931-1939.
8. Kamel F, Saeed C, Qader, S. Magnetic Field Effect on Growth and Antibiotic Susceptibility of *Staphylococcus aureus*. J Al-Nahrain Uni. 2014; 17(3):138-143.
9. Brkovic S, Postic, S, Ilic, D. Influence of the magnetic field on microorganisms in the oral cavity. J Appl Oral Sci. 2016; 23(2):179-186.
10. Al-Musawi M, Al-Rubaye M, Hosseini M. Culture and molecular detection of *Staphylococcus aureus* in dairy products of Ahwaz. AJPS 2014; 14(2):103-107.
11. Morello J, Mizer, H, Granato, P. Laboratory Manual and Workbook in Microbiology Applications to Patient Care. 18th. ed. the McGraw-Hill Companies, Inc., New York. 2006. 95-99 p.
12. Clinical Laboratory Standards Institute. Performance Standard for Antimicrobial Disk Susceptibility Tests. 2018; 31(1):55-60.
13. Romana C, Mária M, Štefánia, V. Modulation of *mecA* Gene Expression by Essential Oil from Salvia sclarea and Synergism with Oxacillin in Methicillin Resistant *Staphylococcus epidermidis* Carrying Different Types of Staphylococcal Chromosomal Cassette *mec*. Inte. J Microbiol. 2016; Article ID 6475837.
14. Livak K and Schmittgen T. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. 2001; 25:402- 408.
15. Al-Ugaili D. Bacteriological and Genetic Studies on Oxacillin Resistant *Staphylococcus aureus* Isolated from Some Hospital in Baghdad City. Dissertation. Al-Nahrain University 2013.
16. Abd Al-Redha A. Impact of Extracellular DNA on Biofilm Thickness and Its Role in Genes Transfer. Thesis. University of Baghdad, 2018.
17. Younan E, Khalawe N, Saleh M, Jaffar, Z. Prevalence of Methicillin Resistance *Staphylococcus aureus* (MRSA) and Methicillin Sensitivity *Staphylococcus aureus* (MSSA) among hospitalized Iraqi patients. Biom Res. 2019; 3(5):154-170.
18. Vaez H, Tabaraei A, Moradi A, Ghaemi, E. Evaluation of methicillin resistance *Staphylococcus aureus* isolated from patients in Golestan province-north of Iran. Afr J Microbiol Res. 2011; 5(4):432-436.
19. Al-Dahbi A and Al-Mathkhury H. Distribution of Methicillin Resistant *Staphylococcus aureus* in Iraqi patients and healthcare workers. Iraqi J Sci. 2013; 54(2):293-300.

20. Abdalla H and Al-Mathkhury H. The Prevalence of methicillin resistant *Staphylococcus aureus* and methicillin resistant *Staphylococcus epidermidis* in AL-Sulaimania city. Iraqi J Sci. 2014; 55(2):386-393.
21. Dibah S, Arzanlou M, Jannati E, Shapouri, R. Prevalence and antimicrobial resistance pattern of methicillin resistant *Staphylococcus aureus* (MRSA) strains isolated from clinical specimens in Ardabil, Iran. Iran J Microbiol. 2014; 6(3):163-168.
22. Alaklobi F, Aljobair F, Alrashod A, Alhababi R, Alshamrani M, Alamin W, Lytvyn L, Alrouki F, Mertz, D. The prevalence of community-associated methicillin-resistant *Staphylococcus aureus* among outpatient children in a tertiary hospital: A prospective observational study in Riyadh, Saudia Arabia. Int J Ped Adol Med. 2015; 2:136-167.
23. Sarvaiya N and Kothari V. Audible sound in Form of music can influence microbial growth, metabolism and antibiotic susceptibility. J Appl Biotechnol Bioeng. 2017; 2(6):48-97.
24. Cenk N and Kekeç Ö. Inactivation of *Escherichia coli* and *Staphylococcus aureus* by ultrasound. The Am Inst Ultrasound Med J. 2014; 6(3):217-277.
25. Kotwal N, Damani J, Damani J. The effect of music on the growth of *Escherichia coli* and *Staphylococcus aureus.* Int J Rec Sci Res. 2016; 7(8):13205-13208.
26. Gu S, Zhang Y, Wu Y. Effects of sound exposure on the growth and intracellular macromolecular synthesis of *E. coli* k- 12. Peer J. 2016; 4:e1920.
27. Sumardi A, Agustrina R, Ekowati C, Oktanisyah R, Madi Hartono M. The effect of magnetic field on antibiotic inhibition for *Escherichia coli* and *Bacillus* sp. J Kedokt Hewan. 2019; 15(2):125-149.
28. Potenza L, Cucchiarini L, Piatti E, Angelini U, Dachà M. Effects of high static magnetic field exposure on different DNAs. Bio-Electro J. 2004; 25:352-365.
29. Fijałkowski K, Peitler D, Żywicka A, Rakoczy R. The effect of rotating magnetic field on enterotoxins genes expression in *Staphylococcus aureus* strains. J Magn. 2016; 21(1):1-7.
30. Meena Sabah Farman, Wissam Khayer Al-Rawi, Maryam I. Salman. The Effect of Exposure to X-Rays on Some Blood Factors in Human Compared with Control. Annals RSCB. 2021; 25(5):11063-11071.