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Effect of Sound Waves as an Alternative Treatment Method of Selective Nosocomial Pathogen

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Abstract: Healthcare-associated infections also known as nosocomial infections, are a leading global health concern, compromising patient safety in hospitals and healthcare facilities. These infections can occur during medical care and even after discharge, impacting both patients and medical staff. Candida albicans is a common cause of responses. Fluconazole is effective against Candida infections, but alternative treatments are needed due to rising antibiotic resistance. This study aims to investigate the impact of Baroque music on nosocomial pathogens, with objectives including collecting pathogens from hospitals, optimizing growth parameters, performing sound wave treatment and assessing growth and susceptibility. The study found that music stimulation reduced cell viability and impacted antibiotic susceptibility in test organisms. Sound waves were shown to influence microbial growth and metabolism, offering a potential alternative to traditional antibiotic treatments. The findings suggest that audible sound, such as music, has the potential to disrupt microbial growth and reduce the spread of nosocomial infections, providing a novel approach to infection control.

Keywords: Nosocomial infections, Candida albicans, Fluconazole, Antimicrobial resistance, Music therapy, Microbial growth, Sound waves.

1. Introduction

Healthcare associated infection, also known as nosocomial infection occurs in hospitals (1). According to the Centre for Disease Control's (CDC), Nosocomial Infections Surveillance (NIS) study, *Candida albicans* and its related species are the 6th most common cause of nosocomial infections (2). Candida infections are generated by the patient's own microflora, whereas Aspergillus infections are caused by inhaling fungal spores from contaminated air during hospital construction or remodeling (3).

Fluconazole has excellent antifungal activity against *Candida albicans* in vitro. Fluconazole is additionally effective against non-albicans fungus species like fungus parapsilosis, and fungus glabrata. Sound waves are classified into three types: infrasound (104 20 Hz),audible sound (20 10-4 Hz) and ultrasound (2 104 1012 Hz).(7). Human ears receive various sound waves and integrate them into a reaction, which is then delivered to the brain, which perceivesstimuli such as music, sound, and noise. As a result, taking into that consideration that, Sound is a mechanical wave that causes a disturbance in the medium, Water is an excellent sound conductor. Cells and bodily fluids are primarilymade up of water. The sum of pure sound frequencies can be described as music.

The influence of audible sound (whether in the form of music or elsewhere) on microbes has received little attention.music has a positive impact on emotions, stress, and the immune system. Neurotransmitters, hormones, cytokines, andpeptides are biochemical molecules that operate as a link between music and its effects [4]. Music also influences theproduction of metabolites, antibiotic sensitivity in organisms. The present research examines the influence of Baroquemusic on particular aspect. The effect of audible sound in the form of music can stimulate the acoustic stress that candisturb growth of microorganisms. To reduce the use of antibiotics and to control the side effects which caused by long term usage of such medication. Hence the aim of this study is to reduce the effect of nosocomial infection with the help of music using the following objectives: To collect the nosocomial pathogens from hospitals, to optimize the microbial growth and to degrade the pathogens using sound wave treatment and to estimate the growth and antimicrobial susceptibility of music treated and untreated pathogens.

2. Objective

The objective of this study is to investigate the influence of Baroque music on nosocomial pathogens, specifically targeting *Candida albicans*, a primary contributor to healthcare-associated infections (HAIs). These infections, whichare often acquired in hospitals and healthcare settings present significant risks to both patients and healthcare workers. Although treatments such as Fluconazole are commonly utilized to address Candida infections, the growing resistance to antibiotics necessitates the exploration of alternative approaches. This study seeks to evaluate whether sound wave stimulation particularly through music can affect microbial growth and antibiotic susceptibility, offering a novel method for infection control. The research will involve the collection of nosocomial pathogens from hospitals followed by the optimization of their growth conditions in a controlled laboratory environment.

To apply sound wave treatment using Baroque music to these pathogens and assess their growth and antimicrobial susceptibility in comparison to untreated controls. Sound waves being mechanical disturbances canpropagate through mediums like water which constitutes a significant portion of cells and bodily fluids, potentially impacting cellular metabolism and microbial growth. This study is based on the hypothesis that sound, particularly inthe form of music, can induce acoustic stress in microorganisms, thereby disrupting their growth patterns and potentially diminishing their resistance to antibiotics. By exploring the effects of music on microbial susceptibility, the research aims to identify a complementary, non-invasive method for reducing nosocomial infections without depending only on antibiotics.

3. Materials And Methods

3.1 SAMPLE COLLECTION

The test organism (*Candida albicans*) was collected from Madras medical college, Rajiv Gandhi Government generalhospital, Chennai 600003.

3.2 Optimization of effect of music treatment on the growth of microorganism

Using Design-Expert software (Version 13, Stat-Ease Inc., MN), a Box-Behnken design was employed to optimize the growth of microorganisms. The design consisted of 13 experimental runs in two blocks, with five replicates at themidpoint. The variables optimized were pH (A), temperature (B), and incubation time (C). A second-order polynomialmodel was generated to analyze the quadratic response surface, estimating main effects, interaction effects, and quadratic effects (9).

3.3 Determination of Microbial cell count

The haemocytometer and glass cover slip were cleaned with approximately 70% ethanol. The counting chambers werethen covered with a glass cover slip. A volume of 10 µl of the sample was pipetted into the haemocytometer using amicropipette. The slide was left undisturbed for 60 seconds to allow the cells to settle. Subsequently, the haemocytometer was examined under a microscope at 100x magnification, focusing on the grid pattern and cell particles. The total number of cells in the four large corner squares was counted, with live yeast cells counted withoutmethylene blue and dead yeast cells counted with it.

3.4 Assessment of minimum inhibitory concentration of treated and untreated microbial culture

The effect of music on antibiotic sensitivity was assessed by inoculating test organisms in fluconazole-containing media at sub-MIC concentrations, with and without music exposure(5).

4. RESULT:

4.1 Optimization of effect of microbial growth on music treatment

The microbial growth was optimized using box-behnken method and the results were showed in 3D interaction plot (Fig 1- Fig 6) and (Table 1) .

Response surface approach was an empirical modelling tool for assessing the link between a set of controlled experimental parameters and observed findings. The empirical model which fits the verified data there by closer the value of R2 to unity. The ANOVA table and regression coefficient analysis for second order polynomial equations forquadratic term.(Table 2) and (Table 3).

Fig 1: Effect of temperature on music treated sample

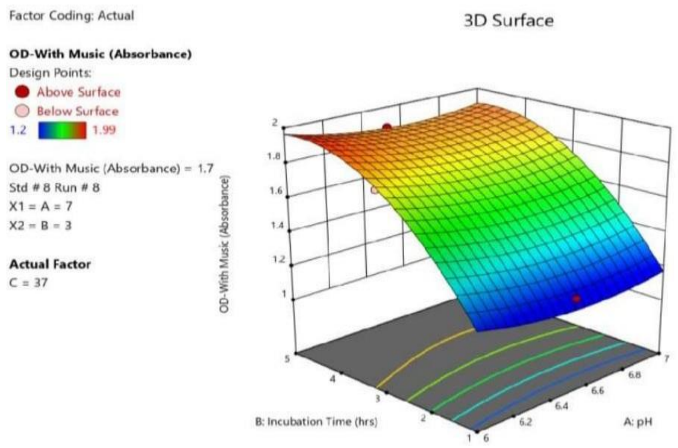


Fig 2: Effect of temperature on music untreated sample

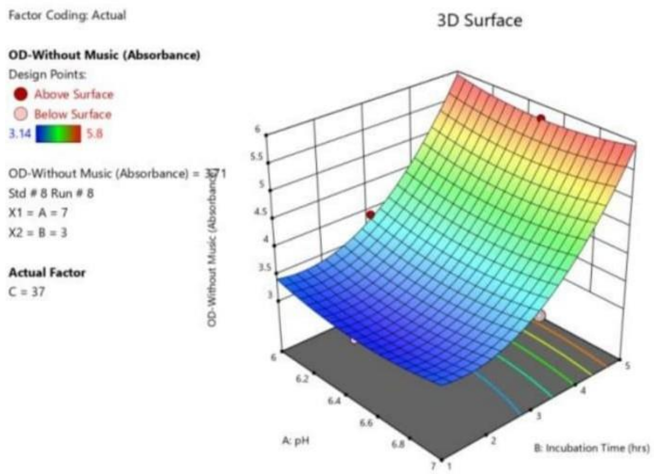


Fig 3: Effect of pH on music treated sample

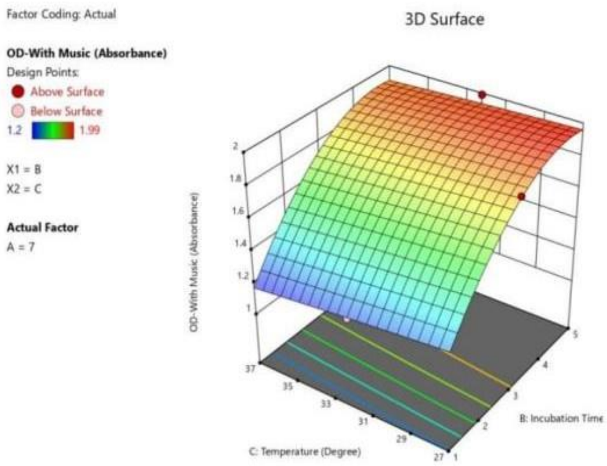


Fig 4: Effect of pH on music untreated sample

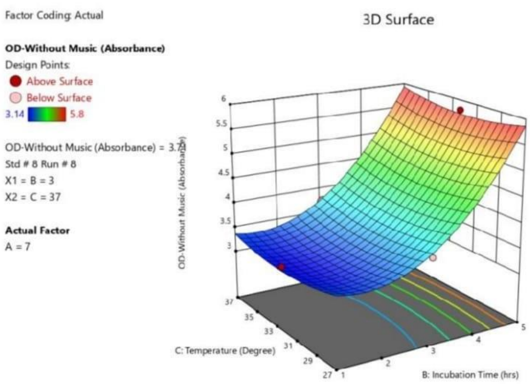


Fig 5: Effect of incubation time on music treated sample

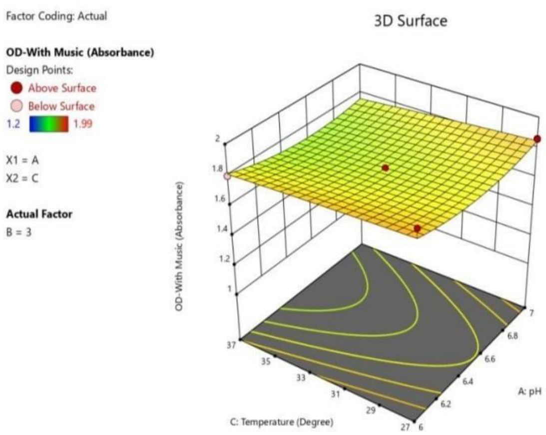


Fig 6: Effect of incubation time on music untreated sample

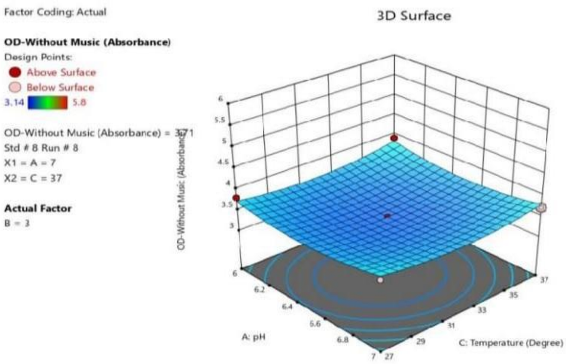


Table 1: Optimization of effect of microbial growth

S.NO	pH(A)	Incubation Time (B) hrs	perature(C)	Response 1 Treated Sample OD (540nm)	Response 2 Untreated Sample OD (540nm)
1	6	5	32	1.98	5.6
2	6	3	37	1.8	3.91
3	6.5	1	37	1.2	3.19
4	7	1	32	1.23	3.29
5	6.5	5	37	1.88	5.75
6	6.5	1	27	1.23	3.18
7	6	1	32	1.21	3.14
8	7	3	37	1.7	3.71
9	6.5	3	32	1.76	3.41
10	6.5	5	27	1.84	5.64
11	6	3	27	1.92	3.81
12	7	3	27	1.86	3.7
13	4	13	7	5	32

Table: 2 ANOVA of the first order regression model of treated sample

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**P-value**	
Model	1.19	9	0.1325	17.89	0.0185	Significant
A- Ph	0.0021	1	0.0021	0.2852	0.6304	
B- Incubation Time	0.9940	1	0.9940	134018	0.0014	
C- Temperature	0.0091	1	0.0091	1.23	0.3483	
AB	0.0000	1	0.0000	0 .0034	0.9573	
AC	0.0004	1	0.0004	0.0540	0.8312	
BC	0.0012	1	0.0012	0.1654	0.7115	
A^2	0.0089	1	0.0089	1.21	0.3525	
B^2	0.1106	1	0.1106	14.93	0.0306	
C^2	0.0000	1	0.0000	0.0019	0.9677	
Residual	0.0222	3	0.0074			
Cor Total	1.21	12				

Table: 3 ANOVA of the first order regression model of untreated sample

Source	Sum of Square	Df	Mean Square	F- value	P- value	
Model	14.29	9	1.59	86.94	0.0018	Significant
A-Ph	0.0002	1	0.0002	0.0110	0.9232	
B-Incubation Time	12.48	1	12.48	683.25	0.0001	
C- Temperature	0.0066	1	0.0066	0.3622	0.5897	
AB	0.0006	1	0.0006	0.0342	0.8650	
AC	0.0020	1	0.0020	0.1109	0.7610	
BC	0.0025	1	0.0025	0.1369	0.7359	
A^2	0.0869	1	0.0869	4.76	0.1172	
B^2	1.66	1	1.66	90.98	0.0024	
C^2	0.0720	1	0.0720	3.94	0.1412	
Residual	0.0548	3	0.0183			
Cor total	14.34	12				

The Model F-value of 86.94 necessitate the model is significant.

The P-values shows less than 0.0500 indicate model terms are significant.The Model F-value of 17.89 necessitate the model is significant.

"Adeq Precision" i.e., signal to noise ratio was 1.85 which indicated an adequate signal. The predicted mean value of

1.76 which represented that the p values <0.05 showed the model is perfectly fit. Consequently, this model canfacilitate design space exploration and negotiation.

The optimum level of 1.76 and 3.14 OD reached at the pH 6.5, 32° C at 3 hr. Optimization of microbial growth by Box- Behnken design showed that the temperature and incubation time played an important role in the growth of *Candida albicans* whereas pH did not showed any noise in the particular optimization.(Table4 and Table 5).

Table 4: Point Prediction of optimization of microbial growth

Two-sided Confidence = 95% Population = 99%

Analysis	redicted Mean	redicted Median	Observed	StdDev	SE Mean	95% CI low for Mean	95% CI high for Mean	95% TI low for 99% Pop	95% TI high for 99% Pop
OD- With Music	1.76	1.76	1.76	0.0860717	0.0745403	1.52278	1.99722	0.937196	2.5828
OD- Without Music	3.79375	3.79375	3.79375	0.135123	0.11702	3.42134	4.16616	2.50204	5.08546

Table 5: Coefficient table of optimization of microbial growth

	Intercept	**A**	**B**	**C**	**AB**	**AC**	**BC**	**A²**	**B²**
OD- With Music	1.76	-0.01625	0.3525	-0.03375	-0.0025	-0.01	0.0175	0.0625	-0.22
p-values		0.6304	0.0014	0.3483	0.9573	0.8312	0.7115	0.3525	0.0306
OD-Without music	3.41	0.005	1.24875	0.02875	0.0125	-0.0225	0.025	0.195	0.8525
p-values		0.9232	0.0001	0.5897	0.8650	0.7610	0.7359	0.1172	0.0024

4.2 Determination of Microbial cell count

The microbial load in the treated and untreated sample was determined using haemocytometer. The methylene dye was used to differentiate the presence of live cells. The microbial cells were counted using light microscope. The microbial cells were counted and its viability was assessed using standard procedure. The treated sample showed 38.1% of viable cells whereas the untreated samples showed 56.4% of viable cells. Hence it demonstrated that the music treatment which acts on the cell wall of the microbes and it thereby loses its viability.(Table 6).

Table 6: Viability percentage of treated and untreated sample

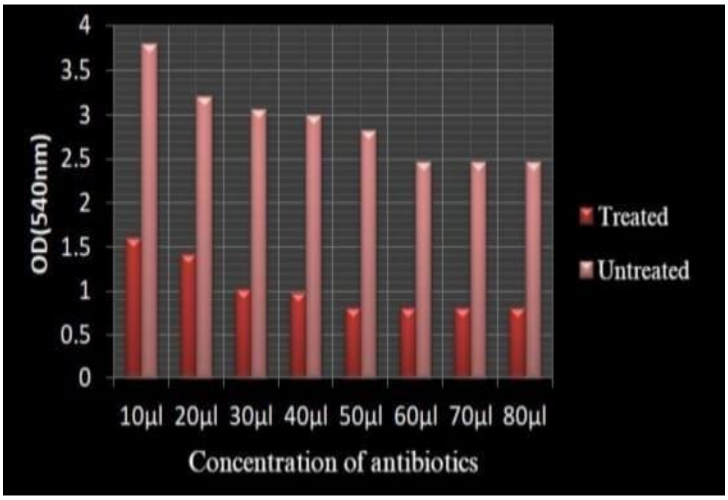
S.No	**Sample treatment**	***Candida albicans***		**Viability %**
		Live cells	**Dead cells**	
1	Treated	42	68	38.181%
2	Untreated	61	47	56.481%

*Untreated – with music treatment , *Treated – without music treatment

4.3 Assessment of Antibiotic susceptibility test

The MIC ranges were observed for different concentration of antibiotics from 10µl to 100 µl. The microbial inhibition was calculated for both treated and untreated sample. The results showed that the 50µg/ml of antibiotic concentration observed with the OD of 2.8 and 0.8 for untreated sample and treated sample respectively. The concentration of antibiotic increases thereby it decreases the microbial growth which observed in the UV-Visible spectrophotometer. The results of sub-MIC revealed that the 50 µg/ml concentration of antibiotics showed breakpoint for the treated sample.(Fig 7).

Fig 7: Optical Density for Antibiotic Concentration



DISCUSSION:

Extensive research has focused on understanding the protective mechanisms of auditory cells against harmful sound vibrations. Music has long recognized for its ability to relieve tension, anxiety, and even pain. More recently, Shaobin *et al* [6] incontestable that *Escherichia coli* bacteria full-grown beneath traditional conditions have their rate of proliferation exaggerated once exposure to frequencies of one, five and ten kHz. E. Ackerman *et al*., as a mechanicalwave, audible sound would cause *Escherichia coli* cells to undergo mechanical stress.(13). Using statistics Niral Sarvaiya and Vijay Gothari discovered that music treatment increased the growth of all test organisms except *S. marcescens*. Under the impact of music, individual cells produced and/or secreted more of the test metabolite [10].Zhao and his colleagues studied the impact of sound stimulation on Chrysanthemum callus growth and observed that it can increase the amount of soluble protein and sugar in the plant callus. As a result, Zhao concluded that soundstimulation at a specific frequency and intensity could increase soluble protein and sugar content, laying the groundwork for cell proliferation and growth [8]. The Heavy Metal (Metallica) genre boosted the spread of *Escherichia coli* and *Staphylococcus aureus* the highest when compared to other music genres. According to Matsuhashi *et al*., sound waves between 6 kHz and 40 kHz can stimulate colony formation in Bacillus carboniphilus [11]. *Lactobacillus spp.* and *Klebsiella spp.* were discovered as sludge-degrading microbes in a previous investigation[12]. Mundus, a German firm, created a low-cost sound system that played Mozart's "The Magic Flute" to biomass- eating bacteria. The sludge was reduced by 1000 cubic metres once a year as a result of the microorganism performedhigher with the music. The wastewater treatment plant saved an estimated 10,000 Euros on the cost of transporting the sludge [12].

5. **CONCLUSION:**

This study successfully shown that audible sound in the form of music can stimulate the acoustic stress that can disturb growth of microorganisms there by changes its cell wall and it metabolism. To reduce the use of antibiotics and to control the side effects which caused by long term usage of such medication. An alternative method can be followed to make an advanced level of killing or inactivating the nosocomial pathogens. *Candida albicans* was acquired from the microbiology department of Madras Medical College, Chennai. Using Design-Expert software, anexperimental design was conducted to optimize the effect of music treatment on the growth of microorganisms by analyzing factors like pH, temperature, and incubation time. Microbial cell count was determined by loading a sampleonto the haemocytometer, allowing cells to settle, and then examining under a microscope to count live and dead yeast cells. The minimum inhibitory concentration of antibiotic-treated and untreated microbial cultures was assessed, with sub-MIC fluconazole concentrations used to test antibiotic sensitivity in the presence and absence of music treatment.

REFERENCES:

1. World Health Organization. The burden of health care-associated infection worldwide. World Health Organization. 2010.
2. Beck-Sague CM, Jarvis WR, National Nosocomial Infections Surveillance System. Secular trends in the epidemiology of nosocomial fungal infections in the United States, 1980-1990. The Journal of infectious diseases. 1993 May 1:1247-51..
3. Sydnor ER, Perl TM. Hospital epidemiology and infection control in acute-care settings. Clinical microbiology reviews. 2011 Jan;24(1):141-73.
4. Gangrade A. The effect of music on the production of neurotransmitters, hormones, cytokines, and peptides:A review. Music and Medicine. 2012 Jan.
5. Sarvaiya N, Kothari V. Effect of audible sound in form of music on microbial growth and production of certain important metabolites. Microbiology. 2015 Mar;84(2):227-35
6. Shaobin G, Wu Y, Li K, Li S, Ma S, Wang Q, Wang R. A pilot study of the effect of audible sound on the growth of Escherichia coli. Colloids and Surfaces B: Biointerfaces. 2010 Jul 1;78(2):367-71.
7. dos Reis Lestard N, Valente RC, Lopes AG, Capella MA. Direct effects of music in non-auditory cells in culture. Noise and Health. 2013 Sep 1;15(66):307.
8. Qin YC, Lee WC, Choi YC, Kim TW. Biochemical and physiological changes in plants as a result of differentsonic exposures. Ultrasonics. 2003 Jul 1;41(5):407-11.
9. Mortazavian AM. Music affects survival and activity of microorganisms. Archives of Advances in Biosciences. 2012 Sep 22;3(4).
10. Shaobin G, Wu Y, Li K, Li S, Ma S, Wang Q, Wang R. A pilot study of the effect of audible sound on the growth of Escherichia coli. Colloids and Surfaces B: Biointerfaces. 2010 Jul 1;78(2):367-71.
11. Norris V, Hyland GJ. Do bacteria sing? Sonic intercellular communication between bacteria may reflect electromagnetic intracellular communication involving coherent collective vibrational modes that could integrate enzyme activities and gene expression. Molecular microbiology. 1997 May;24(4):879-80.
12. Nwambo YP, Kehinde AJ. Sewage and detergent degrading microorganisms in septic tank system. International Journal of Application or Innovation in Engineering & Management. 2013;2(5).
13. Ackerman E. Resonances of biological cells at audible frequencies. The bulletin of mathematical biophysics.1951 Jun;13(2):93-106.
14. Khan HA, Baig FK, Mehboob R. Nosocomial infections: Epidemiology, prevention, control and surveillance.Asian Pacific Journal of Tropical Biomedicine. 2017 May 1;7(5):478-82.
15. Mortazavian AM. Music affects survival and activity of microorganisms. Archives of Advances in Biosciences. 2012 Sep 22;3(4)..
16. Shah A, Raval A, Kothari V. Sound stimulation can influence microbial growth and production of certain key metabolites. Journal of Microbiology, Biotechnology and Food Sciences. 2021 Jan 6;2021:330-4.
17. Ying JL, Dayou J, Phin CK. Experimental investigation on the effects of audible sound to the growth of Escherichia coli. Modern Applied Science. 2009 Mar;3(3):124-7.
18. Ben-Ami R, Olshtain-Pops K, Krieger M, Oren I, Bishara J, Dan M, Wiener-Well Y, Weinberger M, Zimhony O, Chowers M, Weber G. Antibiotic exposure as a risk factor for fluconazole-resistant Candida bloodstream infection. Antimicrobial agents and chemotherapy. 2012 May;56(5):2518-23.
19. Exbrayat JM, Brun C. Some effects of sound and music on organisms and cells: a review. Annual Research& Review in Biology. 2019 Jun 28:1-2.
20. Gangrade A. The effect of music on the production of neurotransmitters, hormones, cytokines, and peptides:A

review. Music and Medicine. 2012;4(1):40-43..

21. Hongbo S, Biao L, Bochu W, Kun T, Yilong L. A study on differentially expressed gene screening of Chrysanthemum plants under sound stress. Comptes rendus biologies. 2008 May 1;331(5):329-33.
22. Hirokawa E, Ohira H. The effects of music listening after a stressful task on immune functions, neuroendocrine responses, and emotional states in college students. Journal of music therapy. 2003 Oct 1;40(3):189-211.
23. Exbrayat JM, Brun C. Some effects of sound and music on organisms and cells: a review. Annual Research& Review in Biology. 2019 Jun 28:1-2.
24. Sarvaiya N, Kothari V. Effect of audible sound in form of music on microbial growth and production of certain important metabolites. Microbiology. 2015 Mar;84(2):227-35..
25. Matsuhashi M, Pankrushina AN, Takeuchi S, Ohshima H, Miyoi H, Endoh K, Murayama K, Watanabe H, Endo S, Tobi M, Mano Y. Production of sound waves by bacterial cells and the response of bacterial cells tosound. The Journal of general and applied microbiology. 1998;44(1):49-55
26. COUNTING OF YEAST CELLS USING HAEMOCYTOMETER [internet] Available from https://www.nexcelom.com/applications/cellometer/cell-counting/counting-yeast-cells-using-a- hemacytometer/