Journal of Nursing Research, Patient Safety and Practice
Vol: 03, No. 02, July-December 2023
ISSN: 2799-1210, DOI: 10.55529/jnrpsp.36.30.41

Research Paper

Comparative study between ear set use in relation with bacterial biofilms causing ear-infections

Muhammed Ozdemir [1*], Asal Aziz Tawfeeq[2], Tunjai Namiq Faiq[3]

[1*]Master student, medical laboratory Techniques Department, Kirkuk Technical College, Northern Technical University, Kirkuk, Iraq.

[2]Associate Professor, Department of Therapeutic Nutrition, Kirkuk Technical College, Northern Technical University, Kirkuk, Iraq.

[3]Consultant Otolaryngologist, Kirkuk Teaching Hospital, Kirkuk, Iraq.

Article Info

Article History:
Received: 15 August 2023
Revised: 24 October 2023
Accepted: 01 November 2023
Published: 18 December 2023

Keywords:
Otitis Media
Ear Infections
Earphones
Bacterial Biofilm
Pathogenic Bacteria
External Auditory Canal

ABSTRACT

Background: Ear infections have recently been identified as a major public health problem in many countries, and are associated with the presence of Gram-positive and Gram-negative bacteria. The ability of these microorganisms to form a biofilm on medical equipment and on headsets has led to concern that there might be a connection between using ear sets by a large number of people and complicated ear infections.
Objective: To assess the correlation between the use of ear sets continuously, their formation of biofilm and the development of ear infections among the people using the headphones.
Methods: This study has been carried out in Kirkuk city from January to May 2023 on 168 participants of both sexes, aged 20-60 years. The participants were divided into Group A (n=120) who used headphones, with 50 male and 70 female, and Group B (n=48) the non-headphones users as control, with 18 male and 30 female. Swabs from ears were taken aseptically and cultured for microbiological and biochemical identification. Biofilm formation was analysed by Congo red agar method. Glutathione reductase (GR), superoxide dismutase (SOD), malondialdehyde (MDA) were measured by ELISA and vitamin D3 was measured using the ichroma™ technique.
Results: Staphylococcus aureus and Pseudomonas aeruginosa were the most common organisms found in ear infections of patients who wore earphones, and male patients were more susceptible. In the ear infection group, reduced vitamin D3 and antioxidant markers (GR, SOD, MDA) levels were found.
Conclusion: The findings indicate that there may be a relationship between oxidative stress, vitamin D3 deficiency and biofilm related ear infections in the ear infection group, especially the headphone group.

Corresponding Author:
Muhammed Ozdemir

Journal of Nursing Research, Patient Safety and Practice (JNRPSP)

Master student, medical laboratory Techniques Department, Kirkuk Technical College, Northern Technical University, Kirkuk, Iraq.
Email: memed.oz93@gmail.com

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1. INTRODUCTION

Much of the microbial flora is found in the skin, mucosa, and ear canals where it aids mammals in a variety of metabolic processes like the creation of ATP and vitamins as well as inherent defensive mechanisms against infections [1] . However, in rare circumstances, the growth of these beneficial microbes might become uncontrolled, resulting in illness [1], [2]. Yet, ear diseases were seen as a serious public health issue in many developing countries with the potentiality to harm patient's psychological and social well-being [3], [4]. In the same context, results of numerous studies revealed that, the majority of people (about 80%) will have at least one episode of otitis media through their lifetime, where 40% of them will have at least six recurrences if they did not treat the infection [3], [4], [5]. Still, the external auditory canal of the ear contains a self-cleaning system in which membranes shed from the tympanic membranes migrate there, but when the natural defenses are compromised, pathogenic organisms such as Pseudomonas aeruginosa, Staphylococcus epidermidis, and Staphylococcus aureus will replace the normal flora causing rapid sever pain [6]. In addition, external factors such as extensive use of earphones or swimming without earplugs has a worse effect on the skin of the external auditory canal [7]. Moreover, numerous studies have shown that teenagers and young adults who use headphones have lower hearing thresholds than those who do not with higher rate of complicated ear infections [8], [9]. Accordingly, found that, Gram-positive and Gram-negative bacteria were both associated with those infections [10]. Also, it was found that, in patients with ear infections, antioxidant and vitamin D3 significantly contributed to the pathogenesis of otitis media with effusion [11], [12]. Since there was no such a study in Kirkuk city evaluating the effect of ear set use association with biofilm formation in patients with ear infections. Therefore, this study was suggested in order to evaluate the relationship between the continuous use of ear set/headphones with biofilm formation and ear infections among handlers of different ages.

2. METHODOLOGY

- **Patients:** This study was carried out in Kirkuk City during January to May 2023 on a total number of (168) participants from both sexes and age range between 20-60 years. Participants were divided into two groups according to ear set use; Group A included (n=120) headphone users with (50 male and 70 female), who attended Kirkuk General Hospital. On the other hand, Group B included non-headphone users of (n=48) participants with (18 male and 30 female) as the control group. Accordingly, study participants signed approval consent and responded to the questionnaire designed by the researchers involved data of age, educational level, smoking status, history of chronic infections, history of ear infection, presence of ear was, and discharge.
- **Sample Collection:** Ear swab samples were collected aseptically under the supervision of Otolaryngologist Consultant from both groups and were immediately cultivated on selective media for microbiological and biochemical diagnostic tests.
- **Biofilm Formation Detection:** The biofilm formation ability by the isolated bacteria was distinguished using the Congo red agar method according to the methodology cited in [12].
- **Serological Tests:** This study involved certain serological test including: the estimation of Human GR, SOD, and MDA antioxidants levels through using of the ELISA technique for all the participants, and the biochemical tests included measuring Vitamin D3 levels by using the ichroma™ technique.

- **Calculation of Results:** Averaging the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Constructing a standard curve with the human GR concentration on the y-axis and absorbance on the x-axis, and drawing a best fit curve through the points on the graph. If samples have been diluted, the concentration read from the standard curve had to be multiplied by the dilution factor. Using some plot software.

3. RESULTS AND DISCUSSION

The external auditory canal of the ear contains a self-cleaning system; when this natural defense is compromised, pathogenic organisms such as Pseudomonas aeruginosa, Staphylococcus epidermidis , and Staphylococcus aureus will replace the normal flora and cause ear infections [13], [14]. While bacterial infections produce rapid, severe pain; the use of ear set might increase the problem. Thus, the total of (168) participants were involved in this study divided into two major groups (Group A and B). Ear swabs were collected from the participants, and microbiological diagnostic test results showed the isolation of multi-species of pathogenic bacteria isolated from Group A participants as illustrated in Figure 1.

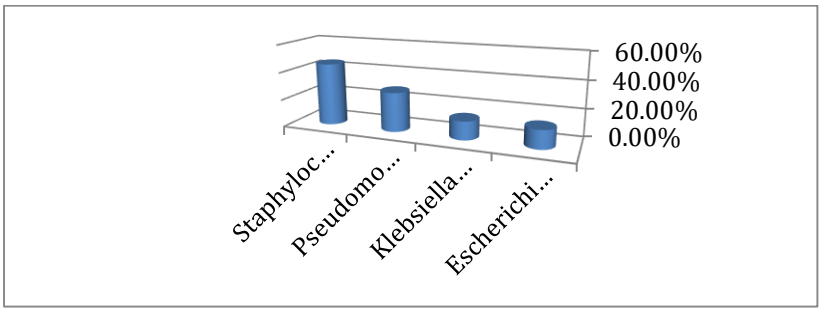


Figure 1. Multi-Species Bacteria Isolated from Group A Participants of the Study

On the other hand, results displayed in Figure 2 below represented the growth ratio of bacteria identified in Group B/ the control group; where they revealed the isolation of 2.08 % of Escherichia coli, 2.08 % of Klebsiella pneumonia, 4.16 % of Pseudomonas aeruginosa, 8.33 % of Staphylococcus aureus respectively .

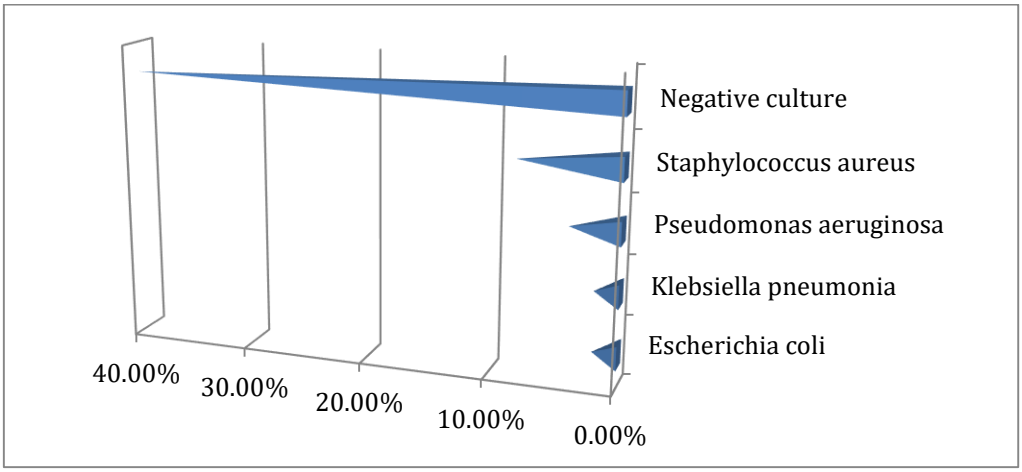


Figure 2. Multi-Species Bacteria Isolated from Group B Participants of the Study

On the other hand, Figure 3 displayed the growth of Staphylococcus aureus on Blood agar and the growth of Staphylococcus aureus on Congo red agar as evidence of biofilm formation.

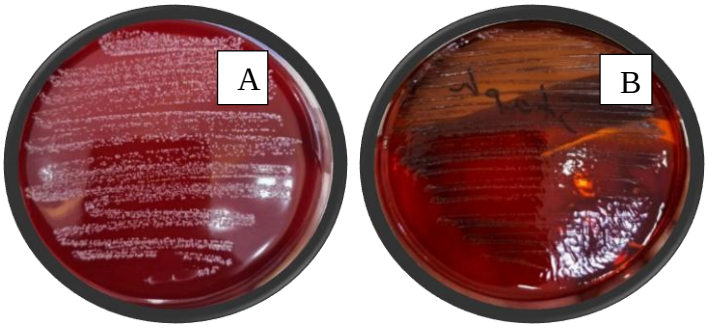


Figure 3. Growth of Staphylococcus Aureus on Blood Agar

Concerning the distribution of age and gender within the study groups the presented study revealed that the greatest rates (48.14 vs. 45.45%) of male and female of patients (head phone users, biofilm positive) respectively were within age group (20-30) years old in comparing to the rest of the age groups within the same study group, while the lowest rates (11.11 vs. 6.06 %) of male and female respectively were found within the age range (50-60) years old of (headphone users, biofilm positive) patients. Similarly, the second study group (headphone users, biofilm negative) patients showed the highest proportions (34.78 vs. 45.94 %) of male and female separately were within age range group (20-30) years old and the lowest rates (13.04 vs. 2.70 %) of male and female patients were within age group (50-60) years old.

The control group of the current study demonstrates 7 (38.88 %) male, and 12 (40%) females within age rage (20-30) years old, the lowest rate 2 (11.11 %) of male patients were within age group (50-60) years old. Table 1.

Table 1. Age and Gender Distribution Among the Study Groups

Age range	**Headphone users with biofilm positive Gender No. (%)**			**Headphone users with biofilm negative Gender No. (%)**			**Control group Gender No. (%)**		
	Male	**Female**	**Total No. (%)**	**Male**	**Female**	**Total No. (%)**	**Male**	**Female**	**Total No. (%)**
20-30	13(48.14)	15(45.45)	28(46.66)	8(34.78)	17(45.94)	25(41.66)	7(38.88)	12(40)	19(39.58)
30-40	7(25.92)	10(30.30)	17(28.33)	5(21.73)	10(27)	15(25)	6(33.33)	8(26.66)	14(29.16)
40-50	4 (14.81)	6(18.18)	10(16.66)	7(30.43)	9(24.32)	16(26.66)	3(16.66)	5(16.66)	8(16.66)
50-60	3(11.11)	2(6.06)	5(8.33)	3(13.04)	1(2.70)	4(6.66)	2(11.11)	5(16.66)	7(14.58)
Total	27(45)	33(55)	60 (100)	23(38.33)	37(61.66)	60(100)	18(37.5)	30(62.5)	48(100)

On the other hand, there was significantly decreasing in the concentration levels of Vit. D3 (19.42 ng/mL) among (headphone users, biofilm positive) patients in comparing to (42.42 ng/mL) within (headphone users, biofilm negative) patients. The difference was highly significant, P=0.000. There was significantly dropping in GR antioxidant concentration level (1124.88 pg/mL) among (headphone users, biofilm positive) patients in matching to (2872.07 pg/mL) for the (headphone users, biofilm negative) patients. The P. Value equal to 0.000, and the results were highly significant. Minimal increment in the concentration level of SOD antioxidant (1.25 U/mL) noticed within (headphone users, biofilm negative) patients in matching to (0.77 U/mL) recorded among (headphone users, biofilm positive) patients.

Regardless that the results were statistically non-significant since the P. value was greater than 0.05. Regarding the MDA concentration level, there was insignificant lowering in their level (382.67 pg/mL) in the (headphone users, biofilm positive) patients group, whereas the (headphone users, biofilm negative) patients documented (479.38 pg/mL). The results were non-significant as shown in Table 2.

Table 2. Comparison between the Patients in Regards to Vit D3, and Anti-Oxidants.

Parameters	Headphone Users, Biofilm Positive Patients	Headphone Users, Biofilm Negative Patients	P. Value
Vit. D3	19.42±0.80	42.42±1.30	0.000
Antioxidants			
GR	1124.88±239.11	2872.07±335.32	0.000
SOD	0.77±0.18	1.25±0.45	0.33
MDA	382.67±35.91	479.38±37.06	0.96
Independent sample t-test was used to analyse the numerical variables, probability (P) value less than 0.05 considered as significant, while P value more than 0.05 considered as non-significant. P value <0.01 highly significant. Mean ± Standard error.			

Vitamin D3 concentration level (19.42 ng/mL) was extremely lowering in (headphone users, biofilm positive) patients' group, in matching to (36.32 ng/mL) that recoded for the (control group). The differences were highly significant. The control group showed greater concentration level of GR (3281.46 pg/mL) in (control group), in matching to (1124.86 pg/mL) that was recorded in (headphone users, biofilm positive) patients' group. The variances were highly significant. In regards to the association of SOD antioxidant levels with the (headphone users, biofilm positive) patients and control group, demonstrate highly significant discrepancies. There was a slight change in the concentration levels of serum MDA (382.67 vs. 544.35 pg/mL) documented among (headphone users, biofilm positive) patients and control group respectively. The difference was non-significant as revealed in Table 3.

Table 3. Relationship between Patients and Control Group on Basis of Vit D3 and Anti-Oxidants

Parameters	Headphone Users, Biofilm Positive Patients	Control Group	P. Value
Vit. D3	19.42±0.80	36.32±1.24	0.000
Anti-oxidants			
GR	1124.86±239.11	3281.46±333.38	0.008
SOD	0.77±0.18	2.01±0.59	0.000
MDA	382.67±35.91	544.35±38.84	0.855
Independent sample t-test utilized to study the numerical variables. Probability (P) value lower than 0.05 considered as significant, while P value greater than 0.05 considered as non-significant. P value <0.01 highly significant. Mean ± Standard error.			

Table 4 exhibit the association between (headphone users, biofilm negative) patients and (control group) in regards to Vit. D3 and (GR, SOD, and MDA) antioxidants levels. It was noticed that vitamin D3 levels recorded a slight difference in their levels (42.42 vs. 36.32 ng/mL) in patients and control groups respectively, and the P. Value was lower than 0.01.

Moreover, GR antioxidant showed higher level (3281.46 pg/mL) in control group when compared to the patient's group that were headphone users but with biofilm negative results, where they recorded (2872.07 pg/mL), but the results were non-significant.

Furthermore, there was an insignificant change in SOD concentration levels (1.25 vs.2.01 U/mL) in patients and control group respectively. The outcomes were non-significant statistically; this was applicable for the concentration levels of MDA as shown in.

Table 4. Comparison between (Headphone Users, Biofilm Negative) Patients and Control Group on the Basis of Vit. D3 And Antioxidants Levels

Parameters	Headphone Users, Biofilm Negative Patients	Control Group	P. Value
Vit. D3	42.42±1.31	36.32±1.24	0.001
Anti-oxidants			
GR	2872.07±335.32	3281.46±333.38	0.664
SOD	1.25±0.45	2.01±0.59	1.109
MDA	479.38±37.06	544.35±38.84	0.898
Independent sample t-test utilized to study the numerical variables. Probability (P) value lower than 0.05 considered as significant, while P value greater than 0.05 considered as non-significant. P value <0.01 highly significant. Mean ± Standard error.			

3.1 Bacterial Growth Among the Study Groups

The presented study revealed that the ratios of bacterial growth among (Group A/headphone users, biofilm positive) patients were (13.33%) for Escherichia coli, (13.33%) for Klebsiella pneumonia, (28.33%) of Pseudomonas aeruginosa, and the greatest rate (45%) of bacterial growth was for Staphylococcus aureus, while the (Group B/the control group) demonstrated (2.08 %) of Escherichia coli, (2.08 %) of Klebsiella pneumonia, (4.16 %) of Pseudomonas aeruginosa, (8.33 %) of Staphylococcus aureus and 40% exhibited no growth. These were supported by Yadav et al., who reported that two important opportunistic bacteria that cause nosocomial and community-acquired illnesses are Staphylococcus aureus and Pseudomonas aeruginosa. Both have been found in biofilm-related infections like chronic middle ear infections and chronic suppurative otitis media [13].

The current study likewise was near to a study conducted in Ramadi city, Iraq, where they found that the two most frequently isolated species were Pseudomonas aeruginosa in (57.5%) of the patients and Staphylococcus aureus in (16.8%) of the cases, this study also showed some disagreement to our study in regards to the percentage of bacterial growth, where they showed as mentioned before the growth proportion of Pseudomonas aeruginosa was higher than the one for Staphylococcus aureus, these variances may be attributed to the differences of sample sizes of both studies and the differences in the age groups, where about 20% of the patients were lower than 10 years old. In which their response to the bacteria is different, since their immune system are not well-developed and response differently to Bacteria [14]. Further studies have revealed that Staphylococcus aureus is the most prevalent cause of otitis media [15], [16]. The presented study also displayed an agreement to a study performed in Duhok city, Iraq, where they found that both Staphylococcus aureus, and Pseudomonas aeruginosa were the most frequent Bacteria involved in otitis media [17]. The biofilm phenotypic trait of Staphylococcus aureus, which permits entrance to the middle ear through the external canal, may be associated to its dominating isolation rate in this investigation.

3.2 Age and Gender Distribution Among the Study Groups

Concerning the distribution of age and gender within the study groups the presented study revealed that the greatest rates (48.14 vs. 45.45%) of male and female of patients (head phone users, biofilm positive) respectively were within age group (20-30) years old in comparing to the rest of the age groups within the same study group, while the lowest rates (11.11 vs. 6.06 %) of male and female respectively were found within the age range (50-60) years old. Similarly, the second study group (headphone users, biofilm negative) patients showed the highest proportions (34.78 vs. 45.94 %) of male and female separately were within age range group (20-30) years old and the lowest rates (13.04 vs. 2.70 %) of male and female patients were within age group (50-60) years old. These were disagreed in regards to the age distribution with a previous study conducted in Northern Cyprus where they found the highest rates of infections settled within age group (65 years old and above), while agreed with our findings in regards to the gender distribution, where they documented the highest rates of infected patients were male [18].

3.3 Comparison between the Patients in Regards to Vit D3, and Anti-Oxidants

The current study revealed that there was significantly decreasing in the concentration levels of Vit. D3 (19.42 ng/mL) among (headphone users, biofilm positive) patients in comparing to (42.42 ng/mL) within (headphone users, biofilm negative) patients. The difference was highly significant, P=0.000. These were close to what was stated by Sohrabpour S et al, who found that patients with otitis media suffered from Vit. D3 deficiency and adding Vitamin D3 to their treatment regimen would help them to relieve from the infection [19], [20]. In addition to that, the findings of the current study was supported by Asher BF, Guilford FT, who found that Otolaryngology patients frequently have Vitamin D deficiency, which can be treated with supplementation. The etiopathology of ear disorders in both adults and children was linked to Vitamin D deficiency [21]. There was significantly dropping in GR antioxidant concentration level (1124.88 pg/mL) among (headphone users, biofilm positive) patients in matching to (2872.07 pg/mL) for the (headphone users, biofilm negative) patients. These were in line with what was reported earlier by, who revealed that GR antioxidant decreased significantly in patients with ear infections [22].

Minimal increment in the concentration level of SOD antioxidant (1.25 U/mL) noticed within (headphone users, biofilm negative) patients in matching to (0.77 U/mL) recorded among (headphone users, biofilm positive) patients. The most potent detoxifying enzyme and antioxidant in a cell is superoxide dismutase (SOD). It is a crucial endogenous antioxidant enzyme that serves as a part of the body's primary defense mechanism against microbial agents, so the decreasing in their concentrations lead to infections and make the host more susceptible to contract infections including ear infections [23].

Regarding the MDA concentration level, there was insignificant lowering in their level (382.67 pg/mL) in the (headphone users, biofilm positive) patients' group, whereas the (headphone users, biofilm negative) patients documented (479.38 pg/mL). This disagreed with Serban R et al., who noticed that patients with ear infections had higher levels of MDA in comparing to healthy control, these differences may be attributed to the variances in the life style in regards to using head phone, and the kinetic responses of MDA in these patients [24].

3.4 Relationship between Patients and Control Group on Basis of Vit D3 and Anti-Oxidants

The presented study also showed that Vitamin D3 concentration level (19.42 ng/mL) was extremely lowering in (headphone users, biofilm positive) patients group, in matching to (36.32 ng/mL) that recoded for the (control group) as shown in Table 4 and likewise it was noticed that vitamin D3 levels recorded a slight differences in their levels (42.42 vs. 36.32 ng/mL) in patients and control groups respectively, and the P. Value was lower than 0.01 as shown in Table 4. These were close to results of a case-control study done by Hosseini S et al., who detected that patients with otitis media had lower Vitamin D levels in matching to healthy control [25]. The control group showed greater concentration level of GR (3281.46 pg/mL) in matching to (1124.86 pg/mL) that was recorded among (headphone users, biofilm positive) patients' group. The variances were highly significant as shown in Table 4. Moreover, GR antioxidant showed higher level (3281.46 pg/mL) in control group when compared to the patient's group that were headphone users but with biofilm negative results, where they recorded (2872.07 pg/mL). In regards to the association of SOD antioxidant levels with the (headphone users, biofilm positive) patients and control group, demonstrate highly significant discrepancies.

Furthermore, there was an insignificant change in SOD concentration levels (1.25 vs.2.01 U/mL) in patients and control group respectively. There was a slight change in the concentration levels of serum MDA (382.67 vs. 544.35 pg/mL) documented among (headphone users, biofilm positive) patients and control group respectively. The difference was non-significant. Table 3. The outcomes were non-significant statistically; this was applicable for the concentration levels of MDA as shown in Table 4. These were in line with what was reported earlier by Ozek H et al., who stated that SOD, and Glutathione levels reduced in patients with ear complications, in comparing to those with no ear infection [26], While our results showed disagreement to Garça MF et al., who found that MDA levels were higher in patients with ear infections in comparing to those with no infection, these differences might be correlated to the differences of bacterial species that caused ear infection and resulted in increased levels of MDA in their study, from the bacterial species detected in our study, and as a result produced high MDA [27].

4. CONCLUSIONS

Staphylococcus aureus and Pseudomonas aeruginosa were more prevalent agents involved in ear infections. Male patients with age range (20-30) were more susceptible patients to contract ear infections. Vitamin D3 concentration levels declined in patients with ear infections along with the Human GR, SOD, and MDA antioxidants levels decreased in patients with ear infections.

Acknowledgments

The authors have no specific acknowledgments to make for this research.

Funding Information

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author Contributions Statement

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Muhammed Ozdemir	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓
Asal Aziz Tawfeeq	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓
Tunjai Namiq Faiq	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓

C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Informed Consent

All participants were informed about the purpose of the study and their voluntary consent was obtained prior to data collection.

Ethical Approval

Not Applicable.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to Cite: Muhammed Ozdemir, Asal Aziz Tawfeeq, Tunjai Namiq Faiq. (2023). Comparative study between ear set use in relation with bacterial biofilms causing ear-infections. Journal of Nursing Research, Patient Safety and Practice (JNRPSP), 3(2), 125-134. https://doi.org/10.55529/jnrpsp.36.30.41

BIOGRAPHIES OF AUTHORS

	**Muhammed Ozdemir,** Muhammed Ozdemir studies at Kirkuk Technical College, Northern Technical University, in Iraq. He is in the Medical Laboratory Techniques Master's program. His focus is on medical lab sciences, clinical microbiology, bacterial infections, and lab tests used for diagnosis. He has worked on bacterial biofilms linked to ear infections. He also looked at how ear-set use connects with bacterial infections. Email: memed.oz93@gmail.com
	**Asal Aziz Tawfeeq**, works as a Professor and Lecturer at Northern Technical University in Iraq. Her training is in biotechnology and molecular biology. She is interested in microbiology and in how bacteria are separated and identified. She also studies molecular diagnosis and uses biotechnology methods in research. Her work includes PCR, DNA extraction, and medical laboratory sciences. She has guided both graduate and undergraduate projects. She has written papers on bacterial infections, antimicrobial sensitivity, and molecular lab testing. Email: drasalaziz@ntu.edu.iq
	**Tunjai Namiq Faiq,** is a consultant otolaryngologist at Kirkuk Teaching Hospital in Kirkuk, Iraq. His work centers on otolaryngology and ear-related conditions. He focuses on finding the cause of ear infections and on how to manage them. He took part in research on bacterial biofilms that relate to ear-set use and ear infections. His clinical input supported work on bacterial pathogens and their role in ear conditions. Email: mutazalharbi84@gmail.com