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Microbial Contamination and Antibiotic Resistance Patterns of Personal Earphones Among University Students in Al-Qadisiyah, Iraq

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ABSTRACT

Background: The widespread use of personal earphones and AirPods as audio devices has increased significantly, particularly among university students. Thus, the concerns about their potential role in carrying microbial contaminants have increased. When the students who share these devices can allow growth of pathogenic microorganisms, particularly when basic hygiene is neglected.

Materials and Methods: About 60 Earphones were gathered from students at Al-Qadisiyah University for both male and female participants. Sterile swabs were used to collect samples from the inner and outer surfaces of the devices. A questionnaire on hygiene practices and sharing habits was performed by participants. The isolates and detection of Staphylococcus aureus of methicillin resistance were confirmed using PCR targeting the nuc and mecA genes as molecular confirmation.

Results: The contamination with microorganisms was distinguished in 93.3% of the samples. S. aureus was the most common isolate (68.3%), also with methicillin-resistant S. aureus (MRSA) found in 8.3% of samples, followed by Pseudomonas aeruginosa (30%), Escherichia coli (25%), and Candida albicans (20%). While mixed growth of bacterial and fungal was present in 28.3% of devices. The identity of *Staphylococcus aureus* isolates and the presence of methicillin-resistant *S. aureus* (MRSA) were confirmed by polymerase chain reaction (PCR).

Conclusion: Earphones that are regularly used by students can lead to opportunistic pathogens. For this reason, routine cleaning and proper hygiene practices may reduce microbial contamination and minimize potential health risks.

Keywords: Earphones, Microbial Isolates, Candida albicans, Drug Susceptibility Testing, Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus (MRSA)

1. Introduction

Earphones have become a fundamental part of daily life routine for several university students, often used for online education, communication, and entertainment as well as online gaming [1,2]. Still, the continued and repetitive use of these devices increases fears about their credibility to harbor microorganisms that may stance health risks [3]. Because of their direct and continued contact with the ear and surrounding skin, coupled with the warm and often moist environment they create, Earphones

could deliver a suitable environment for microbial growth [4,5].

Many studies have shown that private electronic devices, such as headphones and Earphones could carry both bacterial and fungal pathogens, especially when used without proper hygiene practices [6]. Contamination with these organisms could cause serious infection such as external otitis, skin irritation, and in some cases, more severe conditions in immunocompromised individuals [7]. The level of contamination is regularly affected by some factors, such as how often the device is cleaned, the way of its storage, and whether it is shared with others [8].

Many earlier studies have isolated various of pathogenic microorganisms from audio devices Such as Earphone. For instance, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and fungal species like Candida albicans [9]. The contamination with these organisms can be spread via direct contact with shared Earphones [10]. Despite rising global evidence, still there is a clear absence of local studies focusing on microbial contamination of earphones within Iraqi universities particularly in Diwaniyah governorate.

This work focuses on evaluating the microbial contamination of Earphones used by students at Al-Qadisiyah University. The main object is to detect the types of microbial present and their amounts. Furthermore, to discover the causes of Earphones contamination, and focus on possible health risks. Also, this study aims to promote awareness about the importance of routine usage for these devices and suggesting practical recommendations to decrease microbial expansion by hygienic practices.

2. Materials and methods

2.1. Study Setting and Participant Selection

The study included 60 undergraduate (30 males and 30 females) students from multiple science departments at college of science, University of Al-Qadisiyah, Iraq. Their old were from 18-25 years who used personal Earphones consistently for three months. Before sample collection, oral permission was obtained from each participant.

2.2. Sample Collection Procedure

Sterile cotton swabs damped with normal saline to take samples from both inner and outer parts of the Earphones. Swabs were then placed into labeled sterile tubes and promptly transported to the microbiology laboratory to maintain microorganism viability.

2.3. Culture Media and Incubation

Earphone swab samples were cultured onto Nutrient Agar to ensure broad recovery of microorganisms. While Blood Agar was used for precise bacteria with hemolytic activity. MacConkey Agar was accomplished to isolate and differentiation of enteric Gram-negative bacteria. Sabouraud Dextrose Agar was used for fungal growth. Bacterial plates were incubated aerobically at 37 °C for 24–48 hours, whereas fungal plates were maintained at 25–28 °C for up to seven days.

2.4. Identification of Microbial Isolates

After incubation, the colonies were evaluated depend on the size, pigmentation, margin, and texture. Bacterial isolates were subjected to Gram staining followed by Coagulase and IMViC reactions to support initial identification. While fungal isolates were fixed in cotton blue lactophenol and examined microscopically to study the morphology of fungal hyphae and spores. VITEK 2 (bioMérieux, France) was used to enhance diagnostic accuracy after biochemical reactions.

In the present study, isolates identified as S. aureus were classified based on coagulase testing.

However, Coagulase-negative Staphylococci (CoNS) constitute the most part of the normal skin flora and are frequently recovered from specimens obtained from skin or skin-associated surfaces. Nevertheless, the possibility that some CoNS may have been present cannot be completely ruled out.

2.5. Microbial Load Quantification

The number of colonies grown on each plate was counted and expressed as colony-forming units per swab (CFU/swab). The results were analyzed for devices usage according to participant gender and hygiene practices.

2.6. Assessment of Hygiene Behavior

The questionnaire was given to the volunteer students who used earphones frequently from college of science at Al-Qadisiyah university. As to examine the relationship between the usage of earphones and the contamination with various microorganisms depending on routine use and hygiene behavior practices.

2.7. Antimicrobial Sensitivity Testing

Antimicrobial susceptibility was detected by VITEK 2 Compact automated system. The susceptibility was interpreted according to CLSI (2023) criteria [11]. Antibiotics tested included ampicillin, gentamicin, tetracycline, and ciprofloxacin for bacteria. On the other hand, fluconazole and amphotericin B for C. albicans. Quality control strains (S. aureus ATCC 25923, E. coli ATCC 25922, P. aeruginosa ATCC 27853, C. albicans ATCC 10231) were processed alongside samples.

2.8. Molecular Confirmation by PCR

To confirm the identity of S. aureus and detect methicillin resistance PCR was used. DNA was obtained by the boiling method. Primers targeting the nuc gene confirmed the species, while the mecA gene indicated resistance. The reaction was prepared in a 25 µL mixture and amplification was accomplished under standard cycling conditions. The products of PCR were analyzed by agarose gel electrophoresis and then visualized under UV light. To ensure accuracy positive and negative controls were involved.

3. Data Analysis

Data were recorded using Microsoft Excel and analyzed using GraphPad Prism version 10. Microbial loads were expressed as mean ± standard deviation (SD). An independent t-test was performed to compare contamination levels between male and female participants. A p-value of < 0.05 was considered statistically significant.

4. Results

4.1 Prevalence of Microbial Contamination

From 60 samples analyzed, 56 (93.3%) exposed microbial growth as contamination of these devices. On the other hand, only 4 samples (6.7%) were free of detectable microorganisms. This explains that the Earphones can serve as reservoirs of microbes.

4.2 Types of Microorganisms Detected

Bacterial and fungal growth were detected and isolated from the samples. S. aureus was the most identified microbe. This bacterium presents in 41 samples (68.3%) of detected samples. This includes 36 (60.0%) methicillin-sensitive S. aureus (MSSA) and 5 (8.3%) methicillin-resistant S. aureus (MRSA). Other frequently detected microorganisms included P. aeruginosa (18 isolates, 30.0%), E. coli (15 isolates, 25.0%),

and C. albicans (12 isolates, 20.0%). Mixed growth of bacteria and fungi was observed in (17 samples 28.3%) of positive samples (Figure 1). However, no microbial growth was detected in 4 samples (6.7%). The identification of all microorganisms was established by culture, biochemical tests, and (VITEK 2 Compact).

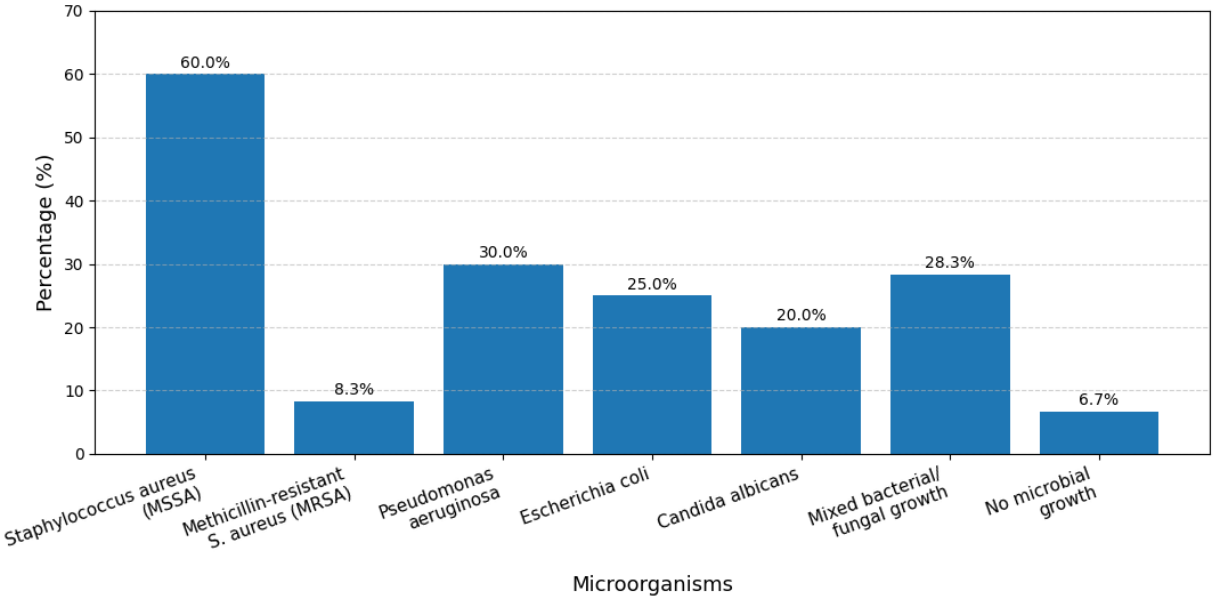

Figure 1. Frequency of Microorganisms Isolated from Earphones of University of Al-Qadisiyah Students (n = 60)

4.3 Comparison by Gender

The microbial load, quantified as colony-forming units (CFU) per swab, was observed to be higher among male students, with an average of 110 CFU, compared to 92 CFU in female students as shown in (Table 1).

Table 1. Comparison of Microbial Contamination by Gender

Parameter	Male Students (n = 30)	Female Students (n = 30)
Mean microbial load (CFU/swab)	110	92
Standard deviation (±SD)	±22.6	±19.4
staphylococcus aureus (MSSA) (%)	60.0% (18/30)	60.0% (18/30)
staphylococcus aureus (MRSA) (%)	10.0% (3/30)	6.7% (2/30)
Escherichia coli (%)	30.0% (9/30)	20.0% (6/30)
Pseudomonas aeruginosa (%)	33.3% (10/30)	26.7% (8/30)
Candida albicans (%)	16.7% (5/30)	23.3% (7/30)
Mixed microbial growth (%)	30.0% (9/30)	26.7% (8/30)
No microbial growth (%)	6.7% (2/30)	6.7% (2/30)
Statistical significance (p-value)	> 0.05 (not significant)	

4.3 Impact of Hygiene Practices

The contributors who never cleaned their Earphones had microbial contamination greater than those who preserved regular cleaning behaviors. Additionally, the contributors who shared their Earphones with others more frequently had opportunistic microorganisms compared to non-sharers with statistical significance ($p < 0.05$) as showed in Table 2.

Table 2. Effect of EarPod Hygiene Practices on Microbial Load and Pathogen Presence

Hygiene Practice	Mean Microbial Load (CFU/swab)	±SD	Pseudomonas aeruginosa (%)	Candida albicans (%)	Statistical Significance
Clean Earphones regularly (n = 25)	74	±18.3	16.0% (4/25)	12.0% (3/25)	[18]
Rarely or never clean Earphones (n = 35)	124	±20.9	40.0% (14/35)	25.7% (9/35)	$p < 0.05$
Do not share Earphones (n = 28)	89	±16.8	21.4% (6/28)	14.3% (4/28)	[19]
Share Earphones with others (n = 32)	117	±21.5	37.5% (12/32)	28.1% (9/32)	$p < 0.05$

4.4 Antimicrobial susceptibility testing

The isolates from personal Earphones have been tested with antimicrobial susceptibility. The results show different forms of sensitivity and resistance among bacterial and fungal species as demonstrated in (Table 3).

Table 3. Antimicrobial Resistance and Sensitivity Rates of Earphones Microbial Isolates

Microorganism	Antimicrobial Agent	Sensitive (%)	Resistant (%)
Staphylococcus aureus (MSSA)	Ampicillin	40	60
	Gentamicin	85	15
Methicillin-resistant S. aureus (MRSA)	Tetracycline	50	50
	Ciprofloxacin	80	20
	Methicillin	0	100
Escherichia coli	Ampicillin	30	70
	Gentamicin	75	25
	Tetracycline	35	65
	Ciprofloxacin	90	10
Pseudomonas aeruginosa	Gentamicin	70	30
	Tetracycline	45	55
	Ciprofloxacin	88	12
Candida albicans	Fluconazole	75	25
	Amphotericin B	90	10
Quality Control	VITEK 2 Compact system	–	–

4.5 Molecular Confirmation of Isolates

To identify *S. aureus* isolates and to determine the existence of MRSA, PCR was applied. On the other hand, to confirm, the isolates produced a 270 bp fragment specific to the *nuc* gene of *S. aureus*. In addition, isolates identified as MRSA produced further 533 bp product conforming the *mecA* gene (Figure 2). To support the validity of the assay, positive and negative controls were used.

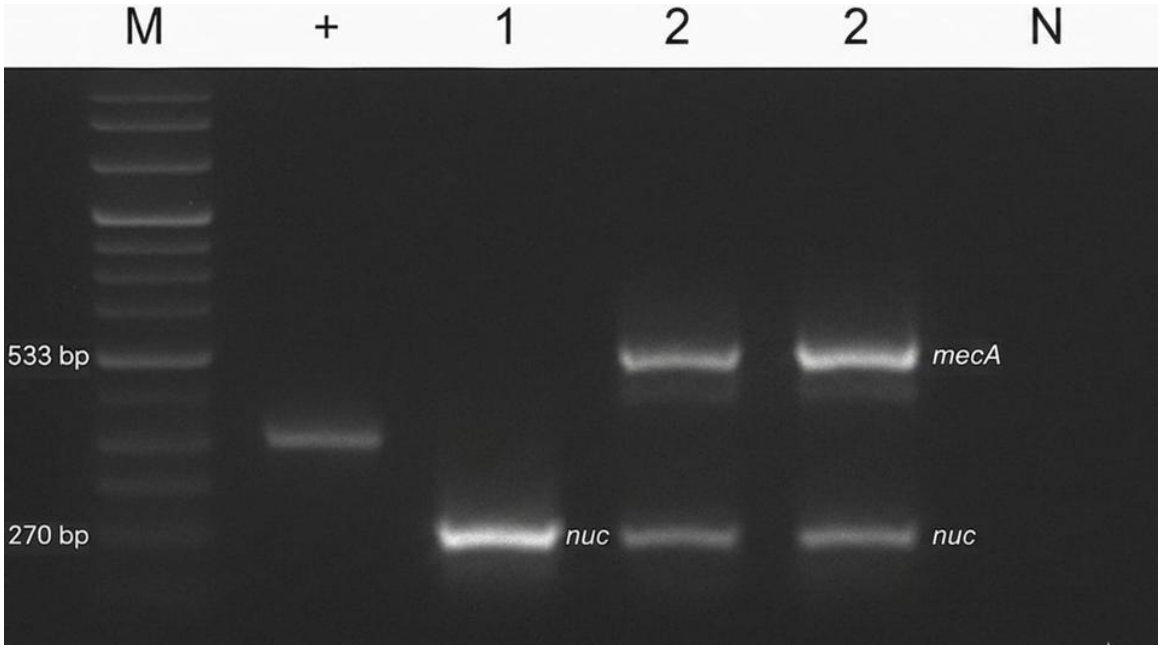

Figure 2. Agarose gel electrophoresis of PCR products from *S. aureus* isolates. Lane M: molecular weight marker; Lane +: positive control; Lane 1: MSSA isolate (*nuc* gene, 270 bp only

5. Discussion

This study reveals a significant microbial problem on personal Earphones among Al-Qadisiyah university students in Iraq. The present work revealed microbes such as S. aureus, P. aeruginosa, E. coli, and C. albicans as the predominant isolates. These results are agreed with global reports. As indicated, personal audio devices act as reservoirs for microbial contamination because of common handling, insufficient cleaning, and shared usage. Similar results were published by Olaitan et al., who stated that these personal tools that used by students carried harmful pathogenic microorganisms related to lake of hygienic routines. Likewise, studies conducted in India reported that earphones and mobile accessories frequently harbor opportunistic bacterial and fungal pathogens [12].

As S. aureus normally lies on skin and nasal passages. This explains the natural colonization of this bacterium transfer to earphones [9]. Although S. aureus was the most isolated organism, the possible existence of CoNS cannot be ignored. In terms of gender, no significant were observed ($p > 0.05$), this suggests similar experience and hygiene behaviors across participants [13,14]. However, the presence of MRSA in males (10%) and females (6.7%) explains the resistant strains of this bacterium in the community [15]. Similar observations were stated in studies from Kuwait and Turkey, where MRSA contamination

was associated with frequently touched personal devices used by university students. On the other hand, variations in the prevalence of E. coli in males and the higher occurrence of C. albicans in females may reflect behavioral and physiological differences [3,16].

The most important key point in this study is the association between hygiene practices and contamination levels. Interestingly, students who did not clean their earphones showed significantly greater microbial loads ($p < 0.05$). However, sharing these tools with each other was strongly associated with opportunistic pathogens such as P. aeruginosa and C. albicans ($p < 0.05$) [17–19]. These findings agree with previous studies relating to poor hygiene and shared earphones used to increase microbial transmission [20,21].

Moreover, more studies stated that humid conditions inside the ear canal with the use of earphones create a constructive environment for microbial survival and biofilm formation, thereby increasing contamination risk [22]. Similar studied about contamination patterns have been reported in Bangladesh, Libya, India, Kuwait, and Turkey, supporting the global relevance of these results [23–26]

Interestingly, antimicrobial susceptibility findings showed clinically significant resistant strains of S. aureus. On the other hand, isolates for same bacterium showed high sensitivity to gentamicin and ciprofloxacin, noted resistance to ampicillin and tetracycline, consistent with global patterns [27]. MRSA isolates (12.2%) exhibited whole resistance to methicillin, this considers as community-associated multidrug-resistant pathogens. While E. coli showed high sensitivity to ciprofloxacin and gentamicin with resistance to ampicillin and tetracycline.

This is because of the reasons for antibiotic misuse and selective pressure [28]. However, P. aeruginosa stayed largely sensitive to ciprofloxacin, moderate resistance to tetracycline was observed. Furthermore, C. albicans showed high sensitivity to amphotericin B, while resistance to fluconazole (25%). In this case, fluconazole can raise clinical concern as it is used in widespread antibiotic [29]. Same study of antimicrobial resistance has been documented worldwide. This reflects the increasing emergence of resistant microorganisms associated with personal devices and environmental contamination [30].

The combination of microbiological approaches including, phenotypic and molecular methods support this study. As PCR identified the nuc gene and the mecA gene of S. aureus. Besides, verified methicillin resistance through detection of that bacterium in agreement with phenotypic findings. This compatibility focuses on the reliability of combining automated VITEK 2 systems and molecular diagnostics to accurately identify resistant strains. Same area of study emphasized that molecular approval advances the accuracy of detecting MRSA as well as enhances the reliability of antimicrobial resistance surveillance the taken isolates [31].

6. Conclusion

This study demonstrates that the frequent uses of earphones among University of Al-Qadisiyah students could harbor potentially harmful microorganisms, including S. aureus, P. aeruginosa, E. coli, and C. albicans. Contamination was related to poor cleaning practices and device sharing. This study approved the importance of regular disinfection and avoiding the sharing of earphones to reduce microbial transmission and potential health risks.

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publication of this article.

The Authors Involved in the Research: The research Sura A. Al-Ganahi, Saja Mahdey Jaber, Hanaa Neamah Abdullah and Robeena Farzand contributed to the research design to analyze the results and write the manuscript, and the authors approved the final version for submission.

Ethical Consideration: The research included samples that were gathered from volunteers without any invasive procedures. According to the guidelines of Al-Qadisiyah university, oral permission was obtained from each participant without formal ethical approval for this type of study.

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