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Biological investigation into antibiotics sensitivity among gram-positive bacteria isolated from headphone and mobile keyboard surfaces

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Abstract
The investigation was carried out among 120 specimens sourced from scientific departments within the Basra Technical Institute throughout January 2022 to January 2023. The study examined microbial proliferation linked to using headphones and mobile keyboards within the institute's premises. Specimens were procured from both headphone surfaces and mobile keyboards and subsequently cultured on Nutrient Agar (NA) and MacConkey Agar (MA), leading to the identification of 28 distinct bacterial strains across the two sources: 60 specimens harvested from mobile keyboards (Ms) and 60 from headphones (Hs). Gram's stain and biochemical assays were employed to validate the isolates' identity. The microbial strains isolated from the above sources comprised 64% gram-negative and 36% gram-positive bacteria (refer to Tables 3, 4, and Figure 3). Further biochemical characterization of the isolates revealed that 8% tested positive for Indole (I), 14% for Voges-Proskauer (VP), 12% for Methyl Red (MR), 12% for Simon's citrate (C), and 10% for Starch hydrolysis (S) (refer to Table 5). Notably, a notable degree of sensitivity was observed, exemplified by zone sizes of 30mm against Streptomycin (S10) and Norfloxacin (NX10), particularly evident in bacterial isolates M9 and E9. Conversely, a comparatively diminished level of sensitivity was noted, with zone Sizes of 11mm against Tetracycline (TE30) and Erythromycin (refer to Table 6). Furthermore, a pronounced level of resistance, reaching 99%, was recorded against Ampicillin (Refer to Table 7).

Keywords: Gram-positive, mobile keyboard, bacteria, headphone

Introduction
The emergence of multidrug resistance in bacterial pathogens has posed a distinctive challenge for antibiotic therapy [1]. Bacteria inhabit both living and inanimate surfaces, yet the widespread presence of microorganisms on numerous public objects, including those in open-air environments, offices, and residential settings, often goes unnoticed. Notably, computer keyboards have gained notoriety for hosting pathogenic microbes. Given their omnipresence, computers have been identified as reservoirs for potentially pathogenic microorganisms, particularly within healthcare facilities. Healthcare- associated infections constitute a substantial burden of morbidity and mortality, with an annual incidence of over 2 million patients, resulting in 90,000 fatalities and healthcare expenditures exceeding $5 billion [4]. Global investigations have documented the isolation of bacteria from computer keyboards, underscoring the colonization of bacterial pathogens on both human hosts and inanimate surfaces. The body of research on nosocomial pathogens elucidates the enduring presence of Gram-positive bacteria, including *S. aureus*, *Enterococcus species*, and *Streptococcus pyogenes*, over extended periods on computer keyboards and mouse [3, 6]. Likewise, numerous Gram-negative bacteria, including *Acinetobacter species*, *Klebsiella species*, *Escherichia coli*, and *Pseudomonas aeruginosa*, demonstrate sustained viability on computer keyboards and mouse surfaces over extended intervals [6]. The variability in contamination levels observed on the surfaces of computer keyboards and mouse is notable. One study, for instance, highlighted this phenomenon by examining 100 keyboards from 29 distinct clinical areas. Remarkably, it found that an overwhelming 95% of the sampled keyboards tested positive for the presence of microorganisms [7]. Based on the prevalence of pathogens detected on computer keyboards (25% of keyboards in healthcare settings harbor pathogenic microorganisms), this rate is twofold higher than that observed on other commonly handled surfaces. This paradox suggests a plausible pathway for the transmission of pathogenic microbes, indicating that interaction with contaminated computer keyboards could facilitate the transfer of potential pathogens onto the hands of healthcare practitioners. Consequently, this scenario may contribute to cross-

contamination among patients, amplifying the risk within healthcare settings. The utilization of earphones has witnessed a surge in popularity as a strategy to mitigate environmental noise and sound pollution. Notably, there has been a marked uptick in earphone usage among young adults, with a concurrent trend of high sharing rates among student populations [8]. Presently, earphones have become indispensable accessories in both personal and professional spheres [9]. The proliferation of earphone usage, particularly among adolescents, coupled with widespread sharing among college students, underscores a pertinent concern regarding health implications [10]. Although earphones are a staple of modern technology for youth, a notable proportion of users are oblivious to the associated health risks [11]. Prolonged utilization of these devices significantly augments the bacterial flora within the ear canal. This phenomenon arises from the elevation of temperature and moisture levels within the ear canal induced by prolonged headphone usage, creating a conducive environment for bacterial proliferation. Empirical investigations have substantiated that sustained earphone usage can precipitate ear canal damage, manifesting as discomfort and hearing impairment [8]. In the ear, species such as Staphylococcus aureus and Pseudomonas bacteria are among the organisms individuals interact with daily without seeing them. Some bacteria may cause diseases, while others are considered beneficial to humans, contributing to various food and pharmaceutical industries [12]. Wearing improper and regularly cleaned earphones may act as a reservoir for colonies of microorganisms that can be transmitted into the ear canal [13]. Moreover, the act of sharing earphones with others can serve as a catalyst for the dissemination of bacteria among individuals. Furthermore, specific earphone designs incorporating rubber or soft sponge coatings have been found to harbor numerous microbes. These microorganisms have the potential to infiltrate the ear canal during earphone usage, posing a potential risk to the user's health [14].

The current investigation has been formulated with the subsequent objectives in mind:

1. To isolate and ascertain the bacterial strains propagated through the interplay involved in using headphones and mobile keyboards.
2. To execute biochemical assays on all isolated bacterial strains.
3. To delineate the isolated bacterial strains' morphological characteristics, classifications, pathogenicity, and associated hazards.
4. To ascertain the potential significance of the frequent use of headphones and mobile keyboards, harboring a substantial microbial load, regarding risk imposition.
5. To explore the antibiotic resistance patterns exhibited by the isolated bacterial strains and to juxtapose their resistance levels.

Materials and Methods

Media used

The media and glassware underwent sterilization within an autoclave under conditions of 15 lb inch-2 pressure and a temperature of 120 °C, maintained for a duration of 20 minutes.

Medium	Composition (g/l)	pH
Soft Agar	Agar: 0.5-0.75	N/A
Nutrient Broth (pH 7.0)	Beef Extract: 3, Peptone: 5, NaCl: 5	7.0
Nutrient Broth (Hi-media, India) (pH 7.4)	Pancreatic Digest of Casein: 5, Yeast Extract: 5, Sodium chloride: 5, Glucose: 2	7.4
Nutrient Agar (g/l)	Peptone: 15, Beef extract: 3, Glucose: 1, Agar: 15	N/A
Mueller Hinton Agar (g/l)	Beef extract: 2, Acid Hydrolysate of Casein: 17.5, Starch: 1.5, Agar: 17.0	N/A
MacConkey Broth (g/l)	Peptone: 20, Lactose: 10, Bile salts: 1.5, Peptone: 5	N/A
Reagents and Buffers	Crystal violet: 0.5-1.0, Iodine: 1.0-1.5, Ethanol: 95%	N/A
Gram Staining Solution	Crystal violet: 0.5-1.0, Iodine: 1.0-1.5, Ethanol: 95%	N/A

S. No	Antibiotic Discs Used	Code	Potency (µg/disc)
1	Ampicillin	AMP	2
2	Tetracycline	TE	30
3	Erythromycin	E	15
4	Azithromycin	AZM	15
5	Streptomycin	S	10
6	Doxycycline	DO	30
7	Narfloxacin	NX	10
8	Amoxicillin	AMX	30

Methods

Collection of head phones and mobile keyboard samples

The acquisition of earphone and mobile keyboard specimens entailed procuring 120 Samples from Basra Technical Institute, subsequently segregated into two cohorts: 60 Samples from earphones (Plate A) and 60 samples from mobile keyboards (Plate B). These cultures underwent incubation for a duration ranging from 24 to 28 hours at a Temperature of 37°C. The quantification of positive cultures facilitated the determination of the overall bacterial load per gram. The specimens were plated on specialized media Such as MacConkey Agar (MA) to facilitate the isolation of individual colonies, which were discerned based on their colonial morphology, Gram staining outcomes, and Adherence to established microbiological methodologies.

Table 1: Head phone samples (Hs) used in the study for bacterial isolation

S. No.	Source	Male Samples	Female Samples	Total Samples
1	Department of Medical Laboratory Technologies	5	5	10
2	Department of Pharmaceutical Technologies	5	5	10
3	Department of Accounting Technologies	5	5	10
4	Department of Nursing Technologies	10	5	15
5	Department of Computer System Technologies	5	10	15
Total		30	30	60

Table 2: Mobile keyboard samples (Ms) used in the study for bacterial isolation

S. No.	Source	Male Samples	Female Samples	Total Samples
1	Department of Medical Laboratory Technologies	5	5	10
2	Department of Pharmaceutical Technologies	5	5	10
3	Department of Accounting Technologies	5	5	10
4	Department of Nursing Technologies	5	5	10
5	Department of Computer System Technologies	5	5	10
6	Department of Civil Technologies	5	5	10
Total		30	30	60

Table 3: Morphological characteristics of bacterial cultures isolation from Headphone samples.

	Characteristic											
Colony	Hs1	Hs2	Hs3	Hs4	Hs5	Hs6	Hs7	Hs8	Hs9	Hs10	Hs11	Hs12
Size	S	S	S	S	m	m	m	m	L	m	S	m
Color	p	y	w	y	w	y	y	w	b	y	y	y
Margin	S	S	S	S	En	Irr	Irr	Irr	S	S	Irr	Irr
Shape	r	r	r	r	r	r	r	r	b	b	b	r
Gram Reaction	NR-	NR-	NR-	NR-	NR-	NR-	NR-	NR-	PR+	PR+	PR+	NR-
KOH	RP+	RP+	RP+	RP+	RP+	RP+	RP+	RP+	NR-	NR-	NR-	RP-

Hs: Headphone samples
S: Small, m: Medium, L: Large
p: Pink, y: Yellow, w: White, b: Brown En: Entire, Irr: Irregular
NR-: Negative Reaction (Gram Reaction), PR+: Positive Reaction (Gram Reaction)
RP+: Positive Reaction (KOH), RP-: Negative Reaction (KOH)

Table 4: Morphological characteristics of bacterial cultures isolation from Mobile keyboard sample

	Characteristic															
Colony	Ms1	Ms2	Ms3	Ms4	Ms5	Ms6	Ms7	Ms8	Ms9	Ms10	Ms11	Ms12	Ms13	Ms14	Ms15	Ms16
Size	L	L	m	S	L	L	S	S	S	m	m	m	m	S	m	
Color	w	w	w	y	w	y	y	w	w	y	y	w	w	y	w	
Margin	Irr	Irr	Irr	re	Irr	re	sm	sm	sm	sm	En	sm	Irr	Irr	En	Irr
Shape	r	r	r	c	r	c	b	r	r	b	b	r	r	c	r	r
Gram Reaction	NR-	NR-	NR-	NR-	PR+	PR+	PR+	NR-	PR+	PR+	PR+	NR-	NR-	PR+	NR-	NR-
KOH	RP+	RP+	RP+	RP+	RN-	RN-	RN-	RP+	RN-	RN-	RN-	RP+	RP+	RN-	RP+	RP+

Ms: Mobile Samples
Size: S (Small), m (Medium), L (Large) Color: w (White), y (Yellow)
Margin: Irr (Irregular), re (Regular), sm (Smooth), En (Entire)
Shape: r (Rod), c (Cocci), b (Bacillus)
Gram Reaction: NR- (Negative), PR+ (Positive)
KOH: PR+ (Positive), PR- (Negative)

Table 5: Biochemical Characteristics of bacterial cultures isolation from mobiles sample

S. NO	Bacterial Isolates	Starch Hydrolysis (S)	Indole (I)	Voges-Proskauer (VP)	Methyl Red (MR)	Citrate Utilization (C)
1	Ms1	NR-	NR-	PR+	NR-	NR-
2	Ms2	NR-	NR-	NR-	NR-	NR-
3	Ms3	NR-	NR-	NR-	NR-	NR-
4	Ms4	NR-	NR-	NR-	NR-	NR-
5	Ms5	NR-	NR-	NR-	NR-	NR-
6	Ms6	NR-	PR+	NR-	NR-	PR+
7	Ms7	PR+	NR-	NR-	NR-	NR-
8	Ms8	PR+	NR-	NR-	NR-	NR-
9	Ms9	PR+	NR-	NR-	NR-	NR-
10	Ms10	PR+	NR-	NR-	NR-	NR-
11	Ms11	NR-	NR-	NR-	NR-	NR-
12	Ms12	NR-	NR-	PR+	PR+	NR-
13	Ms13	NR-	NR-	NR-	NR-	PR+
14	Ms14	NR-	NR-	NR-	NR-	NR-
15	Ms15	NR-	NR-	NR-	NR-	NR-
16	Ms16	NR-	NR-	PR+	PR+	NR-
17	Hs1	NR-	NR-	NR-	NR-	NR-
18	Hs2	NR-	PR+	NR-	NR-	NR-
19	Hs3	NR-	NR-	PR+	PR+	NR-
20	Hs4	PR+	NR-	NR-	NR-	NR-
21	Hs5	NR-	PR+	NR-	NR-	NR-
22	Hs6	NR-	NR-	NR-	NR-	PR+
23	Hs7	NR-	NR-	NR-	NR-	PR+
24	Hs8	NR-	NR-	PR+	PR+	NR-
25	Hs9	NR-	NR-	NR-	NR-	PR+
26	Hs10	PR+	NR-	NR-	NR-	NR-
27	Hs11	PR+	NR-	NR-	NR-	NR-
28	Hs12	NR-	NR-	NR-	NR-	PR+

(PR+) indicates positive reaction;(NR–) indicates negative
Mobile keyboard samples (M), Headphone samples (H)

Table 6: Antibiotic resistance (zone size in mm) of Gram- positive bacteria isolates from Head phone & Mobile keyboard

Bacterial Strains	Ampicillin (2)	Tetracycline (30)	Erythromycin (15)	Azithromycin (15)	Streptomycin (10)	Doxycycline (30)	Narfloxacin (10)	Amoxicillin (30)
M5	R	18	R	R	22	20	R	R
M6	R	20	10	24	23	17	22	10
M7	R	R	12	22	27	R	21	18
M9	R	15	R	21	30	23	30	R
M10	10	R	10	20	12	19	21	13
M11	8	R	11	R	15	15	20	12
M14	R	11	R	19	R	20	10	10
E9	R	22	20	20	16	29	30	19
E10	R	24	21	23	22	R	R	12
E11	R	12	R	20	16	12	25	R
E12	R	10	R	20	R	10	23	R

Strains with zone size > 10mm were considered sensitive and strains with zone size equal to or less than 10mm were considered resistant as per the recommendations of disc manufacturers; values in parenthesis indicate potency of the discs in µg/disc; Ampicillin (AMP2), Tetracycline (TE30), Erythromycin (E15), Azithromycin (AZM15), Streptomycin (S10), Doxycycline (DO30), Norfloxacin (NX10) and Amoxicillin (AMX30).

Table 7: Antibiotic resistance in bacteria isolation from Head phone & Mobile keyboard.

S. NO	Antibiotic discs (mg/disc)	% Resistance
1	Ampicillin (AMP2)	99%
2	Tetracycline (TE30)	33%
3	Erythromycin (E15)	55%
4	Azithromycin (AZM15)	22%
5	Streptomycin (S10)	22%
6	Doxycycline (DO30)	22%
7	Narfloxacin (NX10)	22%
8	Amoxicillin (AMX30)	44%

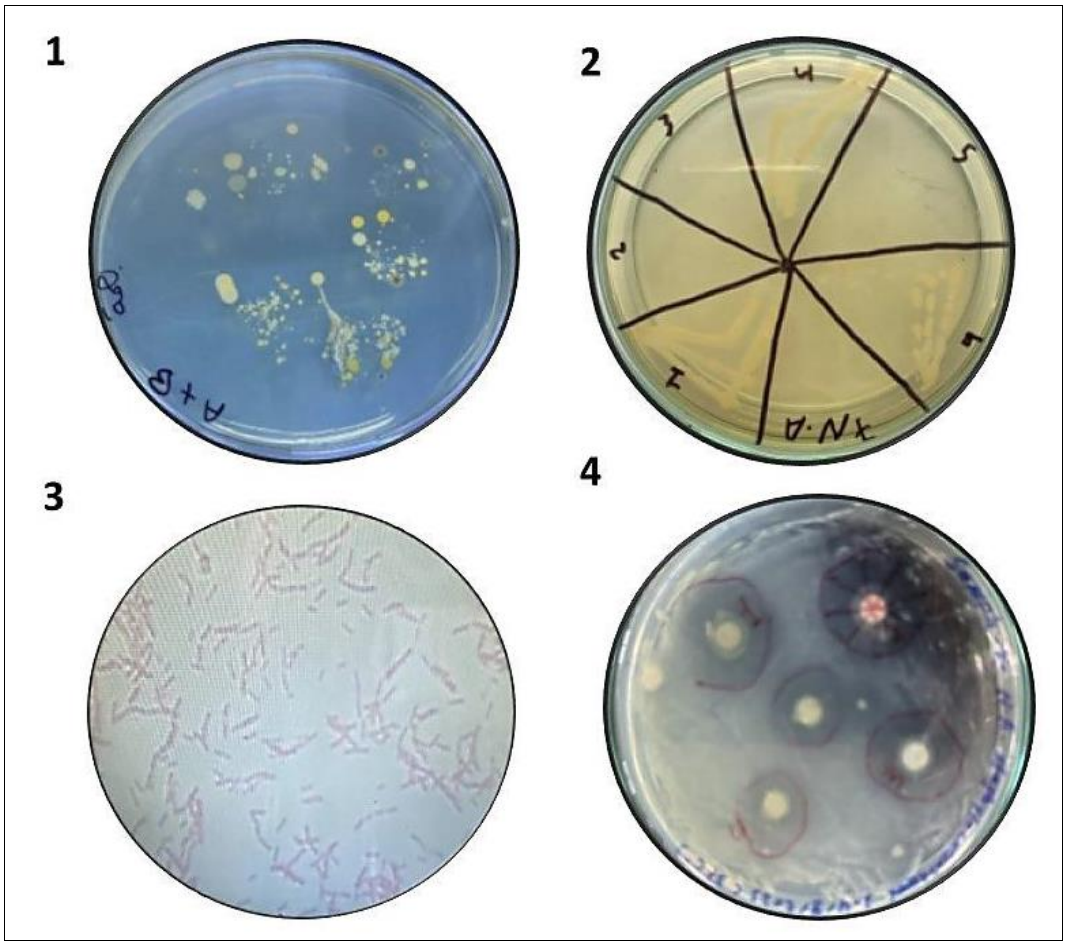

Fig 1: Bacterial colonies on nutrient agar (1), Bacterial isolation by streak method (2), Bacterial isolation under microscopy (3) and Antibiotic resistance (4)

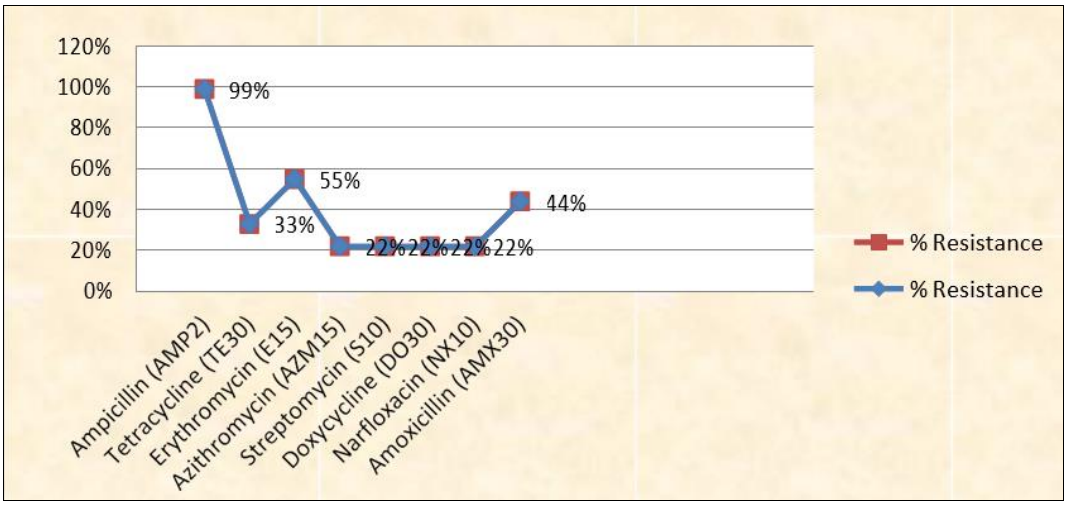

Fig 2: Antibiotic resistance in bacteria isolation from Head phone & Mobile keyboard

Results and Discussion

In total, 28 bacterial colonies were obtained from 120 samples, evenly distributed between 60 samples each from headphones and mobile keyboards (see Figures 1 & 2). Among these, 29 were identified as Gram-positive bacteria via Gram staining, while 17 were classified as Gram-negative. Varied morphologies and quantities were observed among the 28 isolates. Specifically, six were identified as Bacillus, three as cocci, and 19 as rods. A detailed presentation of colony characteristics can be found in Table 3 and Figure 3, encompassing a spectrum of colors from white, yellow, and dark yellow to pink and brown. The morphological, cultural, and biochemical attributes were elucidated by Bergey's Manual of Determinative Bacteriology [7]. Subsequent biochemical analysis was conducted on the isolates. Ten isolates yielded negative results in the Potassium hydroxide test (KOH), while three tested positive for Indole (I). Furthermore, five displayed positive reactions in the Voges-Proskauer test (VP), four exhibited positive outcomes in the Methyl Red test (MR), four demonstrated positivity in Simon's citrate test (C), and seven manifested positive outcomes in Starch hydrolysis (S) (see Table 3). In brief, bacterial suspensions were cultured on Mueller Hinton agar medium, and disks containing antibiotics were introduced. Subsequently, the plates underwent an incubation period of 24 to 28 hours at 37°C. The diametric dimensions of the inhibition zones were utilized to delineate clinical susceptibility or antibiotic resistance. The following antimicrobial agents have been utilized: Ampicillin (AMP2), Tetracycline (TE30), Erythromycin (E15), Azithromycin (AZM15), Streptomycin (S10), Doxycycline (DO30), Norfloxacin (NX10), and Amoxicillin (AMX30) (see Table 4). A total of 10 widely used antibiotics were employed.

The susceptibility and resistance profiles of bacterial strains, particularly Bacilli and Cocci, displayed significant disparity when exposed to widely utilized antimicrobial agents. The susceptibility patterns were evaluated utilizing the disc diffusion technique delineated by Bauer *et al.* (1966) [8]. Substantial levels of susceptibility were evident, as evidenced by zone diameters of 30mm against Streptomycin (S10) and Norfloxacin (NX10) in bacterial isolates M9 and E9. Conversely, diminished susceptibility levels were observed, with zone diameters of 11mm against Tetracycline (TE30) and Erythromycin (E15). Moreover, a heightened resistance level, reaching 99%, was observed against Ampicillin (refer to Table 6 and Figure 4).

Conclusion

In conclusion, regular and persistent use of earphones escalates the probability of bacterial transmission, especially in shared usage among individuals. This escalated risk predisposes individuals to otitis externa, particularly in scenarios involving external ear abrasions. Analogous to the disinfection of stethoscope diaphragms using alcohol, embracing a comparable protocol can mitigate colonization rates and hinder the dissemination of bacterial microflora during earphone interchange. Hence, it is advisable to abstain from sharing earphones altogether or exercise caution when doing so, ensuring thorough cleansing before transferring them to or accepting them from another individual.

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