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Isolation and diagnosis of bacteria resistant to some hand washes found in the markets of Basra, southern Iraq

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Abstract
Sixty samples collection from two Earphones groups: (Group A) 30 student nursing department and (Group B) 30 other random Table (1). The sample was cultured on Nutrient agar and MacConkey Agar. Isolation twelve isolates from all colonies. Biochemical tests and gram test were used to confirm the identification of Table (2). The two types isolates were diagnosed based on their morphology and vitek 2 systems version characteristics Among Include; selected organism 95%probility staphylococcus faecalis, it have bio number 152000622773431 (Ea9, Ea10). 92% probility Staphylococcus vitulinus bio number 100000403263031 Table (3&4). The high level resistance 93% to (Ea 1, 3, 5,7,12) and the low level of resistance 60% to Ea4, Ea6, Ea9 (Table 6).

Keywords: *S. vitulinus*, *S. faecalis*, resistant, markets, Earphones

Introduction
The popular use of earphones is to avoid noise and sound pollution in the surrounding area. Recently, the use of earphones has increased among young adults, and the rate of sharing has increased among students. Frequent use of these headphones leads to a significant increase in the large bacterial population in the ear. Bacteria are among the microorganisms that you encounter every day without actually seeing them. Some of them may lead to diseases, while others are considered beneficial to humans, and they contribute to the food and pharmaceutical industries, as well as contribute to the food and pharmaceutical industries. Some bacteria also have the ability to eliminate organic and inorganic materials [2]. External otitis, which is caused by Staphylococcus aureus, was documented in a nurse who utilized a stethoscope excessively. Smartphones have become commonplace in modern society. As a breeding ground for microbial organisms, these could constitute a global threat to public health via transmission via the microbe [4]. Cell phones are near the body and serve as a reservoir of disease-causing bacteria that allow the transmission of the infective bacteria to multiple different environments. Other healthcare workers (HCWs) and non-healthcare workers (Non-HCWs) may have a distinct role in the transmission of potentially harmful bacteria to the community. Mobile communication devices (MCDs), including cell phones and smart phones, are now commonplace in everyday life. Despite their frequent exposure, the majority of people, including healthcare professionals, often disregarded the potential for these devices to harbor a variety of microbial flora during and after patient inspection. The treatment of MCDs with unhygienic hands and/or their infrequent cleaning can increase the potential for health risks. Many of the harborized bacteria species are detrimental to patients with suppressed immune systems, for whom the infection prevention measures should be taken more seriously. In this article, we explore the importance of maintaining the hygienic nature of mobile devices, specifically in healthcare settings, this will prevent the transmission of nosocomial diseases in patients. Additionally, we discuss methods of addressing the microbial contamination of MCDs that will help maintain good hygienic hand-washing among smartphone users or other mobile communication devices. These methods are capable of providing immediate disinfection of the devices along with a residual effect that is maintained over a long period of time. The development of technology and communication has altered the 21st century entirely, with the introduction of cell phones and smartphones. These phones are associated with the platform of microbes that have been studied recently, this platform provides information on the number and diversity of organisms that are hosted. The utilization of cell phones in the community and in professional fields, including healthcare settings, is considered a possible

vehicle for the spread of microbial diseases. To determine the various types of microbial genetic content present on medical device owned by hospitals. Twenty-six mobile phones belonging to healthcare staff were collected. The DNA was extracted from the stool for the downstream use of next generation sequencing. This procedure was used to profile the microbial population in the intestines. Survey questionnaires were given to the employees to gather information regarding the usage of mobile phones and the behavior of users. Each of the 26 cell phones in this investigation was infested with microorganisms that possessed resistance to antibiotics and pathogenic factors. All 26 mobile phones' microbes and genes were added together, this totalized 11, 163 organisms (5714 bacteria, 675 fungi, 93 protists, and 228 viruses) along with 2096 genes that code for antibiotic resistance and virulent factors. The investigation of medical staff revealed that 46% (12/26) of them used their cell phones in the restroom. Mobile phones are vectors for bacteria and can facilitate the transmission of bacteria and the development of nosocomial diseases worldwide.. As fomites, mobile phones that are not decontaminated may pose serious risks for public health and biosecurity [7]. The emergence of multidrug resistance in bacterial pathogens has presented a unique challenge for antibiotic therapy [8]. Bacterial are colonized on animate and inanimate objects, but most people do not recognize that microorganisms are found on many public objects in the in the open air, in their offices and even in their homes [9]. The Mobil keyboards at helps spread pathogenic microbes. The Mobil keyboards are ubiquitous have contaminated with potentially, pathogenic microorganisms [10, 11]. Many bacteria have been isolated from computer Computers are ubiquitous in the health care centers and have been shown to be contaminated with potentially pathogenic. These healthcare-associated infections are an important cause of morbidity and mortality in hospitals and in each year more than 2 million patients acquire healthcare-associated infections, resulting in 90,000 deaths and healthcare costs that are estimated to exceed $5 billion [8]. Various bacteria is isolated from computers key and in worldwide including developed countries, the bacterial pathogens are colonized on human host and inanimate objects. In different literatures from nosocomial pathogens indicated that Gram-positive bacteria, such as *S. aureus*, Enterococcus species, and Streptococcus pyogenic survive for months on computer keyboards and mice [10, 13]. Many Gram-negative bacteria, such as, Escherichia coli, Klebsiella species, Acenitobacter species and Pseudomonas aeruginosacan survive On computer keyboards and mice surfaces even for months [13]. The degree to which computers keyboards and mice are contaminated is different. One study reported, for example studies from 100keyboards in" 29 clinical areas, 95%" keyboards were positive for microorganisms [14]. The Based on the level of pathogen on Computer keyboards (25%) of keyboards in hospitals carry pathogens microorganisms. the double that of other commonly touched surfaces, Leading to the transmission of pathogenic microbes and that contact with contaminated computer keyboards might serve as a mechanism for contaminating the hands of healthcare health care workforces with potential pathogens, there by leading to cross-contamination of patients Uses of earphones have become popular to avoid noise and sound pollution in the surrounding environment. Recently, there is an increase in the use of earphones among young adults and a high rate of sharing among students [15]. Nowadays, earphones have become one of the most important professional and social life accessories [16] here is a drastic increase in the use of earphones especially among teenagers and has become widespread in sharing among college students [17]. Earphones are a technology used by young people but all today use them without acknowledging the associated health risks [18]. Frequent use of these headphones increases significantly large bacterial flora in the ear. This is because when using headphones the ear constantly, they increase the temperature and moisture of the ear canal. Become this is an ideal environment for bacteria to grow. Studies have shown that continuous use of headphones can cause damage to the ear canal, which can cause damage to the ear canal. It leads to ear pain and hearing loss [15]. In the ear are species *Staphylococcus auerus* and *Pseudomonas* bacteria are among the organisms that you deal with on a daily basis without seeing them some of them may cause diseases and others are considered beneficial to humans, and they contribute to many food and pharmaceutical industries [19]. Wearing earphones that not cleaned properly and regularly may act as a reservoir of microorganism's colonies that can be transmitted into the ear canal [20]. Furthermore, sharing earphones with others can disperse bacteria between people. Besides, certain earphones are coated with rubber or soft sponges that accumulate several microbes. These microbes enter the ear canal when they are used [21]. The final identification was performed with the automated VITEK 2 compact system using GP-ID cards for precise and accurate identification of the isolates at generic and species level.

The Aim 0f study

Isolate and diagnose bacteria that are transmitted through exchange in the use of earphones, as well as to identify their forms and types, infections and the risks they cause, Materials and Methods.

Media Used

Medium / Reagent	Composition
Soft Agar	Agar (0.5-0.8%)
Nutrient Broth (pH 7.0)	Peptone (10 g/L), Beef extract (3 g/L), Sodium chloride (5 g/L), Distilled water (to make 1 L), pH adjusted to 7.0
Nutrient Broth (pH 7.4)	Peptone (10 g/L), Beef extract (3 g/L), Sodium chloride (5 g/L), Distilled water (to make 1 L), pH adjusted to 7.4
Nutrient Agar	Peptone (5 g/L), Beef extract (1.5 g/L), Agar (15 g/L), Distilled water (to make 1 L)
Mueller Hinton Agar	Beef extract (2g/L), Acid hydrolysate of casein (17.5g/L), Starch (1.5g/L), Agar (17g/L), Distilled water (To make 1 L)
MacConkey Broth	Peptone (17 g/L), Lactose (10 g/L), Bile salts (1.5 g/L), Sodium chloride (5 g/L), Neutral red (0.03 g/L), Crystal violet (0.001 g/L), Agar (15 g/L), Distilled water (to make 1 L)
Blood Agar	Trypticase Soy Agar (TSA) base (40 g/L), Defibrinated sheep blood (5-10%), Distilled water (to make 1 L)
Reagents and Buffers	- Gram staining solution: Crystal violet (0.3%), Iodine (1%), Ethanol or acetone (decolorizer), Safranin or fuchsine (0.3-1%), Distilled water
	- KOH: Potassium hydroxide (3-10%)

Hand Wash Uses

S. No.	Hand wash	Made in
1.	Al Awzir Cleaner	Iraq
2.	Fudh	Iraq
3.	C4	Iraq
4.	Savanna	Iraq
5.	Makeen	Iraq
6.	Alif	Iraq
7.	Touti	(Ammaan jordan)
8.	Alen	Iraq
9.	Jofy	(Türkiye)
10.	Visibly Clean	(Türkiye)

Methods

Collection samples

Samples collection from two earphones groups: (Group A) 30 student nursing department and (Group B) 30 other random Table (1). The colonies incubation for (24 to 28 hour at 37 ºC). The number of positive cultures were used to determine the total bacterial count /g. Cultures were plated on nutrient agar and selective media (MA) MacConkey Agar to isolate single colonies Figure 1., which were determines by correlating colonial appearance, Gram stain, and standard microbiological procedures Figure 2.

Results and Discussion

The total of 12 colonies bacterial cultures were isolated from 60 samples divide into (Group A) 30 student nursing department and (Group B) 30 other random (Figure1). Twelve identified as 3 bacteria give Gram positive on the basis of Gram's staining Out of 9 Gram negative bacteria. The bacteria were distinguished in several forms and in different numbers among the 12 bacteria that were isolated, and the results showed that 9were rod shape & 3 were Cocci characteristics of the isolates are presented in (Table 3& Figure 3), The color of colonies was generally white, yellow and dark yellow, pink and brown The morphological, cultural and biochemical characteristics in accordance with Bergey's Manual of Determinative Bacteriology [10]. The plates were incubated for (24 to 28 hour at 37 ºC). Diameters of the growth inhibition zone were used to Identify, the clinical resistance/susceptibility in to hand wish (Ea1-12). Total of 12 common antibiotics were used hand wish resistance /sensitivity profiles of bacterial strains namely (Bacilli ssp, Cocci), against common antimicrobial varied considerably, the antibiotic sensitivity behavior of the strains were determined by disc diffusion method [11]. A high level of sensitive show zone size in mm (15mm) against W6 It appears in bacterial isolates Ea4 and low level of sensitive show zone size in mm (11mm) against Ea3and Ea7. The high level resistance 100% to Ea1, Ea6, Ea10, Ea9, Ea12 (Table 5 & Fig 4). Microbial bacteria isolated from the sources was the following; gram negative bacteria (64%) and gram positive (36%) and the (Table 3 & 4& Fig 3). The high level resistance 93% to A4 and the low level of resistance 60% to A10 (Table 6).

Table 1: Earphones samples (Group A) and (Group B) used in the study for bacterial isolation

S. No.	Samples collection			
	Department of nursing technologies (Student Group A)		Other random (Other student Group B)	
1.	Male	Female	Male	Female
2.	5	5	5	5
3.	5	5	5	5
4.	5	5	5	5
5.				
Total	30		30	

*(Group A) 30 student nursing department and (Group B) 30 other random

Table 2: Morphological characteristics of bacterial cultures isolation from Earphones.

	Colony	Ea1	Ea2	Ea3	Ea4	Ea5	Ea6	Ea7	Ea8	Ea9	Ea10	Ea11	Ea12
Morphological Characteristics	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	c	c	c	r
	Gram reaction	GR-	GR-	GR-	GR-	GR-	GR-	GR-	GR-	GR+	GR+	GR+	GR-
	KOH	R+	R+	R+	R+	R+	R+	R+	R+	R-	R-	R-	R+

(*H) Mobiles samples Indicates positive (R+) reaction & negative (R-) reaction. B (bacillus), c (Cocci), r (Rod)
(*m) Medium, (*L) Large, (*S) small,
(*y) yellow, (w) white, (b) brown color, (*P) pink color
(*Irr) Irregular, (*re) regular, (*S) smooth, (*En) Entire

Table 3: Biochemical characteristics of *S. faecalis* isolated from Earphones.

Organism Quantity			
Selected Organism: Entercoccus faecalis			
Source: Collected			
Comments			
Identification Information	Analysts Time:	2.82 hours	Status: Final
Selected Organism	95% Probability	Entercoccus faecalis	
	Blonumber:	152000622773431	

ID Analysis Messages																
Biochemical Details																
2	AMY	+	4	PIPLC	-	5	dXYL	-	8	ADHI	+	9	BGAL	-	II	AGLU	+
13	APPA	-	14	CDEX	+	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	-	29	TyrA	+	30	dSOR	+	31	URE	-	32	POLYB	+	37	dGAL	-
38	dRIB	-	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	+	52	e4AN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	-	39	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Table 4: Biochemical characteristics of *S. vitulinus* isolated from Earphones.

Organism Quantity:
Selected Organism : Staphylococcus vitulinus

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:** 6.83 hours	**Status:** Final
Selected Organism	92% Probability **Staphylococcus vitulinus** **Bionumber:** 100000403263031	
ID Analysis Messages		

Biochemical Details																	
2	AMY	+	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	+	31	URE	-	32	POLYB	-	37	dGAL	-
38	dRIB	+	39	lLATk	+	42	LAC	-	44	NAG	-	45	dMAL	+	46	BACI	-
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Table 5: Resistance of the bacterial isolates From Earphones.

SO. N	**Hand lotion**									
	W1	**W2**	**W3**	**W4**	**W5**	**W6**	**W7**	**W8**	**W9**	**W10**
Ea1	R	R	R	R	R	R	R	R	R	R
Ea2	R	R	R	R	8	8	8	7	R	R
Ea3	R	R	R	R	R	R	R	R	R	R
Ea4	10	9	9	R	R	R	R	8	7	10
Ea5	R	R	R	R	R	R	R	R	R	R
Ea6	R	R	R	R	8	R	8	7	R	8
Ea7	R	R	R	R	R	R	R	R	R	R
Ea8	12	R	R	R	9	R	14	8	R	R
Ea9	R	R	11	14	10	15	10	7	10	7
Ea10	11	10	8	R	R	R	11	R	R	10
Ea11	13	R	R	R	14	13	R	R	9	R
Ea12	R	R	R	R	R	R	R	R	R	R

Table 6: % Percent resistance of the bacterial isolates From Earphones.

SN.0	**Hand wish**	**% Resistance**
1	Al Awzir Cleaner	66%
2	FUDAH	80%
3	C4	80%
4	Savannah	93%
5	Makeen	66%
6	Alif	80%
7	Touri	66%
8	Alen	66%
9	Jofy	80%
10	Visibly Clean	60%

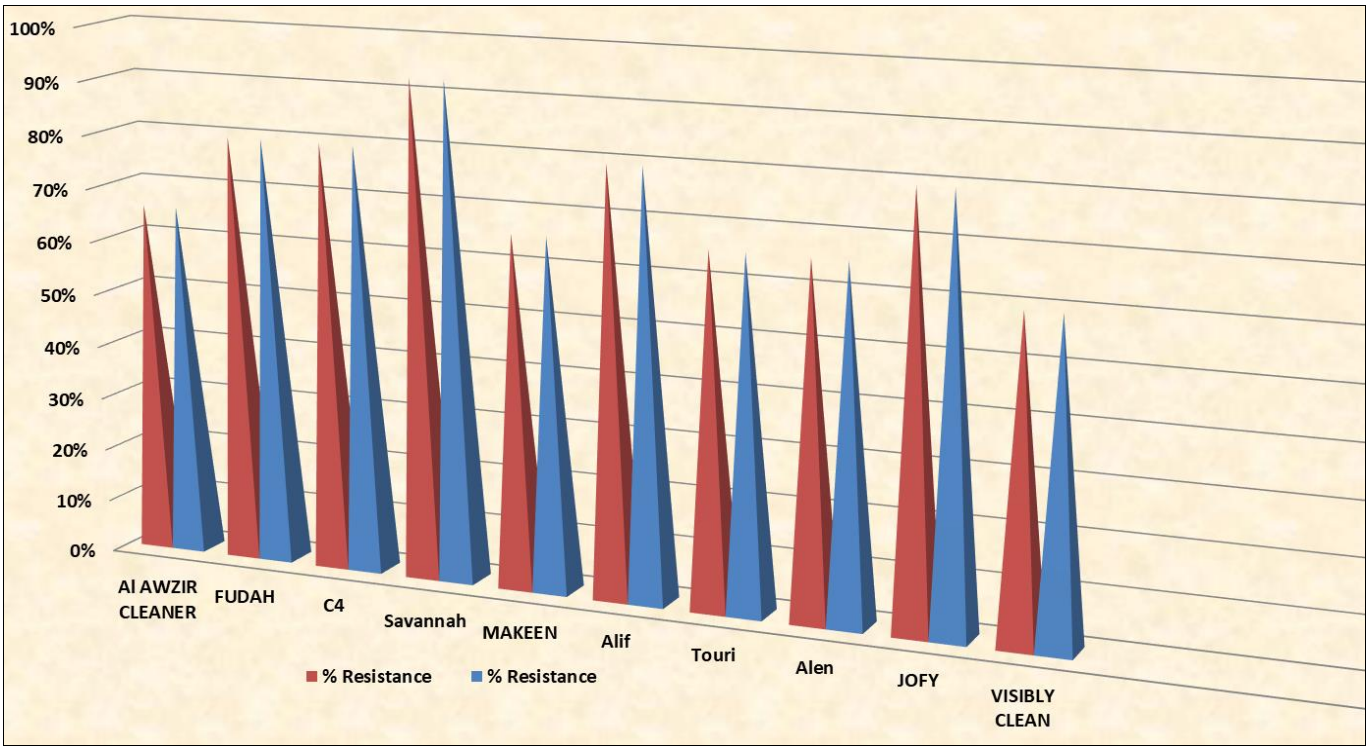

Fig 1: Antibiotic resistance in bacteria isolation from earphones

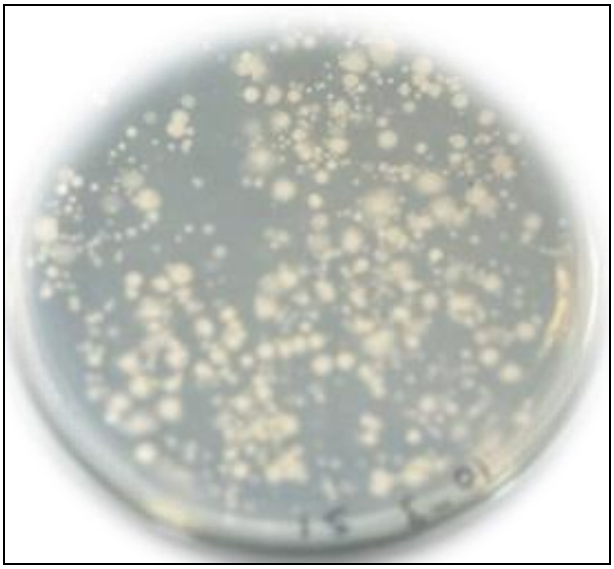

Fig 2: Bacterial colonies on nutrient agar

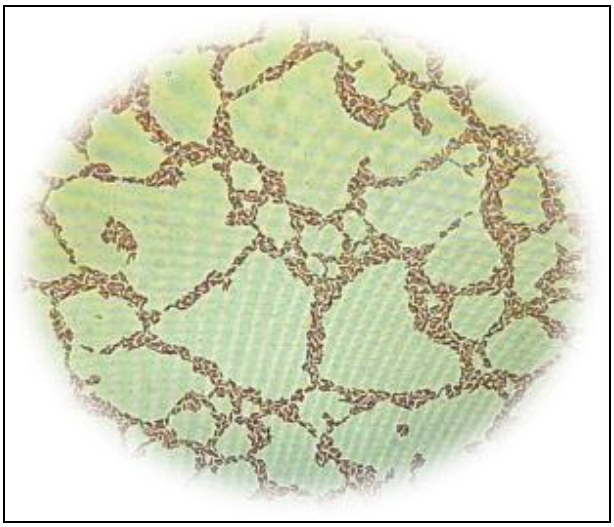

Fig 3: Bacterial gram stain

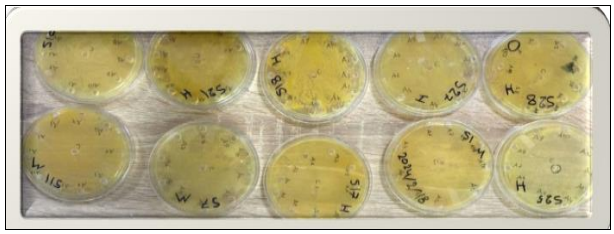

Fig 4: Show hand wish resistance in bacterial isolates from Earphone

Conclusion

It can be concluded that bacterial transfer does increase with frequent and continuous use and the chance of it being transferred is high while people tend to share Earphone while listening to music. This may increase a chance of otitis external as well especially if there is any abrasion in the external ear. *The cleansing of the diaphragms of the stethoscopes with alcohol has been shown to reduce the colonization rate and the same procedure can be adopted to prevent transmission of colonization flora from one to another when headphones are exchanged. It is suggested therefore not to share earphones or to share with caution, like cleaning it before giving it to or taking it from someone else.

Recommendation

Taking care of the cleanliness of the hands and ear and not underestimating the danger that can be caused by the multiple types of microbes scattered in the mobile and headphones. Make sure to use a good type of hand wash trying to reduce the excessive use of mobile or the placement of earphones for a long time.

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