

Determination of bacterial and fungal flora from headphones used by students in Rivers State University, Nigeria

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ABSTRACT

Headphones are increasingly used by students and may harbor microorganisms due to frequent handling, skin contact, and environmental exposure. This study aimed to isolate and characterize bacterial and fungal flora associated with headphones used by students at Rivers State University. Ten (10) headphone swab samples were collected and cultured using Nutrient Agar and Sabouraud Dextrose Agar following standard microbiological techniques. Antibiotic and antifungal susceptibility tests were performed on the bacterial and fungal isolates, respectively. Total heterotrophic bacterial counts ranged from 4.3×10^5 to 2.07×10^6 CFU/ml of swab suspension, while total heterotrophic fungal counts ranged from 1.2×10^4 to 4.1×10^4 SFU/ml. Twenty-three (23) bacterial isolates belonging to six genera and eleven (11) fungal isolates belonging to four genera were identified. The bacterial isolates comprised *Staphylococcus* spp. 7 (30.4%), *Bacillus* spp. 9 (39.1%), *Listeria* spp. 2 (8.7%), *Enterococcus* spp. 1 (4.3%), *Corynebacterium* spp. 2 (8.7%), and *Micrococcus* spp. 2 (8.7%). The fungal isolates comprised *Aspergillus* spp. 4 (36.4%), *Rhizopus* spp. 4 (36.4%), *Penicillium* spp. 1 (9.1%), and *Candida* spp. 2 (18.2%). *Bacillus* spp. showed the highest susceptibility to levofloxacin 9 (100%) and erythromycin 8 (88.9%), but resistance to ceftazidime 7 (77.8%) and cefuroxime 6 (66.7%). *Staphylococcus* spp. showed high susceptibility to ciprofloxacin 7 (100%) and azithromycin 6 (85.8%), but resistance to ceftazidime 4 (57.1%) and rifampicin 3 (42.9%). Antifungal susceptibility was assessed using a modified agar-well diffusion assay with fluconazole, ketoconazole, and itraconazole at concentrations of 500 mg, 250 mg, 125 mg, and 62.5 mg. *Rhizopus* spp. showed resistance to fluconazole 3 (75%) and itraconazole, but susceptibility to ketoconazole 2 (50%). *Candida* spp. showed resistance to itraconazole 2 (100%) but susceptibility to fluconazole 1 (50%) and ketoconazole 1 (50%). The findings indicate that headphones can serve as potential vehicles for bacterial and fungal microorganisms. Although the small sample size limits conclusions regarding population prevalence, the presence of potentially pathogenic microorganisms highlights a possible public health risk among students. Improved hygiene practices and a non-sharing policy are recommended for headphone users.

Keywords: Headphones, ear infection, antibiogram, public health.

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INTRODUCTION

Headphones are electronic devices worn over, on, or in the ears, allowing users to listen to audio privately without disturbing others (Merriam-Webster, 2024). Locally, the terms headphones, earphones, and earpieces are often used interchangeably to describe devices worn over, on,

or in the ears.

With the increasing global advancement of digital technology, personal electronic devices, particularly headphones, have become important accessories for communication, entertainment, and professional

activities. Sharing headphones is also common among individuals, often without consideration of the potential health risks. Studies have shown that frequent and prolonged use of earphones can increase bacterial growth in the ear canal. For instance, research conducted at Manipal University in India found that individuals who frequently used earphones had higher bacterial counts in their ears than infrequent users (Mukhopadhyay et al., 2008).

The human ear harbors a variety of microorganisms, including bacteria, fungi, and viruses, some of which constitute the normal flora and contribute to a balanced microbial community. However, under certain conditions, such as increased humidity, microbial populations may become opportunistic and contribute to infections, including otitis externa (swimmer's ear), fungal ear infections, and skin-related conditions. Shared headphones may act as fomites, facilitating the transfer of potentially pathogenic microorganisms between users and increasing the risk of infection (Stroman et al., 2001).

There has been an increasing trend in the use and sharing of earphones among young adults, particularly students (Mukhopadhyay et al., 2008). Studies of the human environment have demonstrated the presence and colonization of fomites by microorganisms (Bures et al., 2000). Humans come into daily contact with various fomites, which may serve as potential vehicles for the transmission of bacterial and fungal pathogens (Eguia and Chambers, 2003). The use and sharing of headphones or earplugs have been implicated as possible predisposing factors for external ear canal infections because they may increase the temperature and humidity of the ear canal, cause skin abrasion, and provide a vehicle for introducing microorganisms into the canal (Abbinaya and Bharathi, 2012).

The external auditory canal is normally colonized by commensal bacteria that constitute the resident flora of the ear. These organisms are predominantly non-pathogenic and aerobic and include *Staphylococcus* species such as *Staphylococcus auricularis*, *Staphylococcus epidermidis*, *Staphylococcus capitis*, and, in some cases, *Staphylococcus aureus*; coryneform bacteria such as *Turicella otitidis* (Stroman et al., 2001); alpha-haemolytic *Streptococcus* species (Mukhopadhyay et al., 2008); and *Pseudomonas aeruginosa* (Clark et al., 1997).

Research on the microbial implications of prolonged earphone use among young adults remains limited (Ansari and Mohammadpoorasl, 2016). Although previous studies have examined microbial contamination of personal electronic devices and earphones, evidence directly linking headphone contamination to microbial transmission or ear infections remains limited. Therefore, this study aimed to determine the cultivable bacteria and fungi associated with headphones used by students at Rivers State University, Port Harcourt, and to describe their antimicrobial screening profiles.

MATERIALS AND METHODS

Study area

The study was carried out at Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria. Samples were collected from students using headphones on the university campus.

Sample collection

A total of ten (10) students who used headphones were selected for the study, and swab samples were aseptically collected from their headphones. The sampled devices included headsets, earpieces, and earbuds. Each device was sampled using a sterile swab, and the specimens were transported to the Microbiology Laboratory at Rivers State University for analysis. Information on frequency and duration of use, sharing practices, cleaning practices, headphone material, and history of ear infection was not systematically incorporated into the sampling design and, therefore, could not be evaluated as potential confounding factors.

Sample preparation

Moistened sterile swabs were used to collect material from the surfaces of the headphones and were transferred into tubes containing 9 ml of sterile normal saline. The tubes were shaken vigorously to obtain the swab suspension. Serial ten-fold dilutions were prepared up to 10^{-3} . Aliquots (0.1 ml) of the 10^{-3} dilution were inoculated onto Nutrient Agar for bacterial enumeration, while an appropriate dilution was inoculated onto Sabouraud Dextrose Agar (SDA) for fungal enumeration. The inoculum was spread evenly using a sterile bent glass rod. The bacterial plates were incubated at 37 °C for 24 hours, while the fungal plates were incubated for 72–120 hours. The resulting counts were reported as colony-forming units per millilitre (CFU/ml) for bacteria and spore-forming units per millilitre (SFU/ml) for fungi in the swab suspension.

Inoculation and isolation of microorganisms

The swab suspensions were serially diluted in sterile normal saline and inoculated onto Nutrient Agar and Sabouraud Dextrose Agar. Nutrient Agar plates were incubated at 37 °C for 24 hours, while SDA plates were incubated for 72–120 hours. Following incubation, discrete colonies were counted, and viable counts were calculated based on the dilution factor and plated volume. Results were expressed as CFU/ml for bacteria and SFU/ml for fungi in the swab suspension. The sampled

surface area was not standardized; therefore, contamination density per unit surface area was not calculated.

Characterization and identification of pure cultures

Discrete colonies were counted and purified by subculturing onto freshly prepared Nutrient Agar or Sabouraud Dextrose Agar using the streak-plate technique. Pure cultures were obtained and incubated at 37 °C for 24 hours for bacterial isolates and for 72–120 hours for fungal isolates. The isolates were subsequently characterized using cultural and microscopic methods.

Characterization and identification of bacterial and fungal isolates

Bacterial isolates were characterized based on colony morphology, including colony form, elevation, margin, surface texture, size, and colour or pigmentation. Microscopic examination was performed using Gram staining to determine Gram reaction and cellular morphology.

Fungal isolates were characterized based on colony morphology and microscopic examination following staining with Lactophenol Cotton Blue (LPCB).

Antimicrobial susceptibility profile

Antimicrobial screening of the bacterial isolates was carried out using the Kirby–Bauer disc diffusion method. Purified isolates were suspended in 2 ml of sterile normal saline and adjusted to approximately a 0.5 McFarland turbidity standard. The standardized suspensions were inoculated onto Mueller–Hinton Agar and tested using the antibiotic discs employed in the study: rifampicin (20 µg), ceftazidime (30 µg), streptomycin (30 µg), azithromycin (10 µg), ciprofloxacin (10 µg), erythromycin (30 µg), levofloxacin (20 µg), gentamicin (10 µg), cefuroxime (30 µg), and amoxicillin (20 µg). The plates were incubated at 37 °C for 18–24 hours. Zones of inhibition were measured and interpreted with reference to CLSI (2024), where applicable to the organism–antibiotic combination.

Preparation of antifungal drugs and SDA

Antifungal activity was assessed using a modified agar-well diffusion method. Fresh fungal cultures were grown on Sabouraud Dextrose Agar, and a standardized inoculum was prepared from each isolate. The fungal suspension was inoculated onto sterile SDA plates, after which wells were aseptically bored. The prepared antifungal agents fluconazole, ketoconazole, and itraconazole, were added at 0.1 ml per well at the

experimental concentrations reported in the study (500, 250, 125, and 62.5 mg). The plates were incubated at 25–30 °C for 3–5 days, after which the inhibition zones were examined and measured in millimetres.

Because the agar-well diffusion procedure used in this study is not a standardized CLSI susceptibility-testing method, the observed responses were treated as experimental screening results rather than definitive clinical resistance or susceptibility categories.

The antifungal procedure was informed by the general principles of antifungal susceptibility testing described by the Clinical and Laboratory Standards Institute (CLSI). CLSI M38 and M44 describe standardized methods for specific fungal groups and testing formats; however, the present study employed a modified agar-well diffusion procedure. Accordingly, the observed inhibition responses are reported as assay-specific screening findings, and their interpretation is limited to the experimental conditions used in this study.

Data analysis

Microbial counts, isolate frequencies, and antimicrobial screening results were summarized descriptively using counts, percentages, and ranges. Given the sample size of ten devices and the absence of a comparator group, no inferential statistical tests were performed. Therefore, the findings cannot be generalized to the wider student population.

RESULTS

Total heterotrophic bacterial and fungal counts

Table 1 presents the total heterotrophic bacterial count (THBC) and total heterotrophic fungal count (THFC) obtained from the headphone swab suspensions. THBC ranged from 4.3×10^5 to 2.07×10^6 CFU/ml, while THFC ranged from 1.2×10^4 to 4.1×10^4 SFU/ml.

Characterization of bacterial and fungal isolates

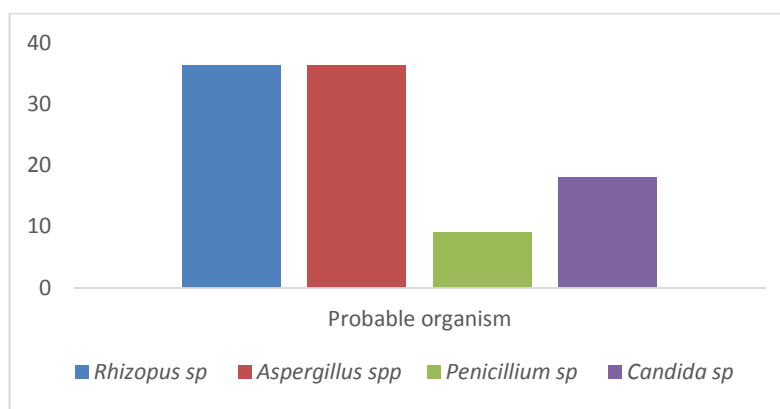
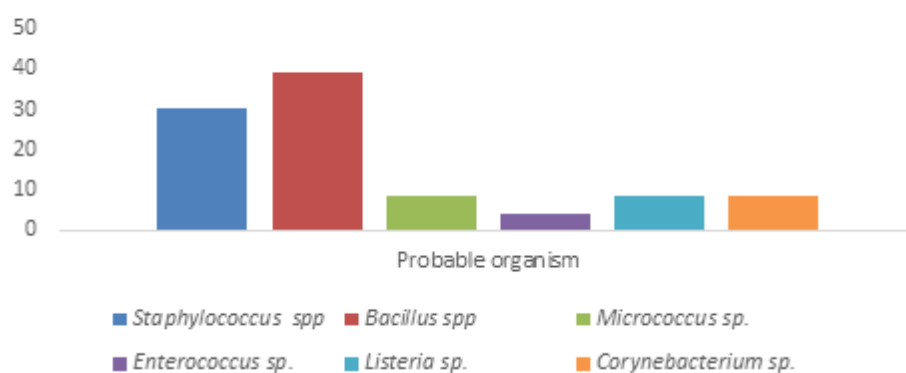
A total of 23 bacterial and 11 fungal isolates were obtained and characterized using cultural, biochemical, and microscopic observations. The bacterial isolates were classified into six genera: *Staphylococcus* spp., *Micrococcus* spp., *Listeria* spp., *Bacillus* spp., *Enterococcus* spp., and *Corynebacterium* spp. The fungal isolates were classified into four genera: *Aspergillus* spp., *Candida* spp., *Penicillium* spp., and *Rhizopus* spp.

Percentage occurrence

The percentage occurrence of the bacterial and fungal isolates is presented in Figures 1 and 2. Among the

Table 1. Total bacterial and fungal count.

Samples	Total heterotrophic bacterial count (CFU/ml of swab suspension)	Total heterotrophic fungal count (SFU/ml of swab suspension)
1	1.18×10^6	4.1×10^4
2	7.2×10^5	2.6×10^4
3	5.0×10^5	2.3×10^4
4	4.3×10^5	2.1×10^4
5	7.8×10^5	1.6×10^4
6	7.3×10^5	1.2×10^4
7	6.9×10^5	1.9×10^4
8	2.07×10^6	1.3×10^4
9	1.36×10^6	2.2×10^4
10	1.09×10^6	1.5×10^4

**Figure 1.** Percentage occurrence of fungal isolates from all samples.**Figure 2.** Percentage occurrence of bacteria isolates from all samples.

bacterial isolates, *Bacillus* spp. had the highest percentage occurrence, with 9 (39.1%), followed by *Staphylococcus* spp. with 7 (30.4%). *Enterococcus* spp. had the lowest occurrence, with 1 (4.3%).

Among the 11 fungal isolates, *Rhizopus* spp. and *Aspergillus* spp. had the highest percentage occurrence, with 4 (36.4%) isolates each, followed by *Candida* spp. with 2 (18.2%). *Penicillium* spp. had the lowest

occurrence, with 1 (9.1%) isolate.

Antifungal screening of fungal isolates

The experimental antifungal screening responses of the fungal isolates obtained from the headphones are presented in Table 2. Fluconazole, ketoconazole, and

Table 2. Antifungal screening/susceptibility profile of fungi isolated from headphones.

Organisms	Fluconazole n(%) 500(mg)			Ketoconazole n(%) 500(mg)			Itraconazole n(%) 500(mg)		
	R	S	I	R	S	I	R	S	I
<i>Rhizopus</i> sp. (4)	3(75)	1(25)	0(0.0)	1(25)	2(50)	1(25)	3(75)	1(25)	0(00)
<i>Candida</i> sp. (2)	1(50)	1(50)	0(0.0)	1(50)	1(50)	0(00)	2(100)	0(00)	0(00)
<i>Penicillium</i> (1)	0(0)	1(100)	0(00)	0(00)	1(100)	0(00)	0(00)	0(00)	1(100)
<i>Aspergillus</i> sp. (4)	3(75)	1(25)	0(0.0)	1(25)	2(50)	1(25)	1(25)	0(00)	3(75)
		250(mg)			250(mg)			250(mg)	
<i>Rhizopus</i> sp. (4)	3(75)	1(25)	0(00)	1(25)	2(50)	1(25)	3(75)	1(25)	0(00)
<i>Candida</i> sp. (2)	2(100)	0(00)	0(00)	1(50)	0(00)	1(50)	2(100)	0(00)	0(00)
<i>Penicillium</i> (1)	0(00)	1(100)	0(00)	0(00)	1(100)	0(00)	0(00)	0(00)	1(100)
<i>Aspergillus</i> sp. (4)	3(75)	0(00)	1(25)	2(50)	1(25)	1(25)	4(100)	0(00)	0(00)
		125(mg)			125(mg)			125(mg)	
<i>Rhizopus</i> sp. (4)	3(75)	0(00)	1(25)	2(50)	0(00)	2(50)	3(75)	1(25)	0(00)
<i>Candida</i> sp. (2)	2(100)	0(00)	0(00)	1(50)	0(00)	1(50)	2(100)	0(00)	0(00)
<i>Penicillium</i> (1)	0(00)	1(100)	0(00)	0(00)	1(100)	1(100)	1(100)	0(00)	0(00)
<i>Aspergillus</i> sp. (4)	4(100)	0(00)	0(00)	2(50)	1(25)	1(25)	4(100)	0(00)	0(00)
		62.5(mg)			62.5(mg)			62.5(mg)	
<i>Rhizopus</i> sp. (4)	4(100)	0(00)	0(00)	1(25)	0(00)	3(75)	4(100)	0(00)	0(00)
<i>Candida</i> sp. (2)	2(100)	0(00)	0(00)	1(50)	0(00)	1(50)	2(100)	0(00)	0(00)
<i>Penicillium</i> (1)	0(00)	0(00)	1(100)	0(00)	0(00)	1(100)	1(100)	0(00)	0(00)
<i>Aspergillus</i> sp. (4)	4(100)	0(00)	0(00)	1(25)	0(00)	3(75)	4(100)	0(00)	0(00)

Keys: R/S/I = Resistant/Intermediate/Susceptible, n(%) = number of isolates (percentage).

itraconazole were tested at the experimental concentrations reported as 500, 250, 125, and 62.5 mg. The numerical distributions presented in Table 2 represent assay-specific screening responses under the conditions used in this study.

For *Rhizopus* spp., at 500 mg, three isolates were resistant to fluconazole and one was susceptible. For ketoconazole, one isolate was resistant, one was intermediate, and two were susceptible. For itraconazole, three isolates were resistant, one was intermediate, and one was susceptible. At 250 mg, three *Rhizopus* spp. isolates were resistant to fluconazole, one was intermediate, and one was susceptible. For ketoconazole, one isolate was resistant, one was intermediate, and two were susceptible, while three isolates were resistant to itraconazole, one was intermediate, and one was susceptible. At 125 mg, three isolates were resistant to fluconazole, one was intermediate, and none was susceptible. Two isolates were resistant to ketoconazole and two were intermediate, while three were resistant to itraconazole and one was susceptible. At 62.5 mg, all four *Rhizopus* spp. isolates were resistant to fluconazole and itraconazole, while one isolate was resistant to ketoconazole.

For *Candida* spp., at 500 mg, one of the two isolates was resistant to fluconazole and ketoconazole, while the other was susceptible to both drugs. Both isolates were resistant to itraconazole. At 250 mg, both isolates were resistant to fluconazole and itraconazole, while one was resistant to ketoconazole and the other was intermediate. At 125 and 62.5 mg, both isolates were resistant to fluconazole and itraconazole, while one was resistant to

ketoconazole and the other was intermediate.

At 500 and 250 mg, the single *Penicillium* sp. isolate was susceptible to fluconazole and ketoconazole but showed an intermediate response to itraconazole. At 125 mg, the isolate was susceptible to fluconazole and ketoconazole but resistant to itraconazole. At 62.5 mg, it was resistant to itraconazole but showed intermediate responses to fluconazole and ketoconazole.

For *Aspergillus* spp., at 500 mg, three isolates were resistant to fluconazole and one was susceptible. For ketoconazole, one isolate was resistant, one was intermediate, and two were susceptible. For itraconazole, one isolate was resistant and three showed intermediate responses, with none susceptible. At 250 mg, three isolates were resistant to fluconazole and one was intermediate. Two isolates were resistant to ketoconazole, one was susceptible, and one was intermediate, while all four isolates were resistant to itraconazole. At 125 mg, all four isolates were resistant to fluconazole and itraconazole, while two were resistant to ketoconazole. At 62.5 mg, all four *Aspergillus* spp. isolates were resistant to fluconazole and itraconazole; one was resistant to ketoconazole, while three showed intermediate responses.

Antibacterial susceptibility of bacterial isolates

The antibacterial susceptibility profiles of bacterial isolates obtained from headphones used by students at Rivers State University are presented in Tables 3-5.

For *Bacillus* spp. (n = 9), two isolates were resistant to

Table 3. Susceptibility pattern of *Bacillus* spp. (9) and *Staphylococcus* spp. (7).

Antibiotics	Concentration (ug)	<i>Bacillus</i> spp. (9)			<i>Staphylococcus</i> spp. (7)		
		R n(%)	S n(%)	I n(%)	R n(%)	S n(%)	I n(%)
Amoxil	20	2(22.2)	5(55.6)	2(22.2)	4(57.1)	2(28.6)	1(14.3)
Ciprofloxacin	10	0(00)	8(88.9)	1(11.1)	0(00)	7(100)	0(00)
Erythromycin	30	0(00)	8(88.9)	1(11.1)	1(14.3)	5(71.4)	1(14.3)
Levofloxacin	20	0(00)	9(100)	0(00)	0(00)	7(100)	0(00)
Gentamycin	30	2(22.2)	7(77.8)	0(00)	3(42.9)	4(57.1)	0(00)
Cefuroxime	30	6(66.7)	3(33.3)	0(00)	3(42.9)	3(42.9)	1(14.2)
Rifampicin	20	4(44.5)	2(22.2)	3(33.3)	3(42.9)	3(42.9)	1(14.2)
Ceftazidime	30	7(77.8)	0(00)	2(22.2)	4(57.1)	3(42.9)	0(00)
Streptomycin	30	1(11.1)	6(66.7)	2(22.2)	2(28.6)	5(71.4)	0(00)
Azithromycin	10	1(11.1)	8(88.9)	0(00)	1(14.2)	6(85.8)	0(00)

Keys: R - Resistant, I - Intermediate, S - Susceptible, n(%) = number of isolates (percentage).

Table 4. Susceptibility pattern of *Micrococcus* sp. (2) and *Enterococcus* sp. (1).

Antibiotics	Concentration (ug)	<i>Micrococcus</i> sp. (2)			<i>Enterococcus</i> sp. (1)		
		R n(%)	S n(%)	I n(%)	R n(%)	S n(%)	I n(%)
Amoxil	20	0(00)	2(100)	0(00)	1(100)	0(00)	0(00)
Ciprofloxacin	10	0(00)	2(100)	0(00)	0(00)	1(100)	0(00)
Erythromycin	30	0(00)	2(100)	0(00)	0(00)	1(100)	0(00)
Levofloxacin	20	0(00)	2(100)	0(00)	0(00)	1(100)	0(00)
Gentamycin	30	0(00)	2(100)	0(00)	1(100)	0(00)	0(00)
Cefuroxime	30	2(100)	0(00)	0(00)	1(100)	0(00)	0(00)
Rifampicin	20	2(100)	0(00)	0(00)	1(100)	0(00)	0(00)
Ceftazidime	30	2(100)	0(00)	0(00)	1(100)	0(00)	0(00)
Streptomycin	30	0(00)	2(100)	0(00)	1(100)	0(00)	0(00)
Azithromycin	10	1(50)	0(00)	1(50)	1(100)	0(00)	0(00)

Keys: R - Resistant, I - Intermediate, S - Susceptible, n(%) = number of isolates (percentage).

Table 5. Susceptibility pattern of *Listeria* sp. (2) and *Corynebacterium* sp. (2).

Antibiotics	Concentration (ug)	<i>Listeria</i> sp. (2)			<i>Corynebacterium</i> sp. (2)		
		R n(%)	S n(%)	I n(%)	R n(%)	S n(%)	I n(%)
Amoxil	20	1(50)	1(50)	0(00)	1(50)	0(00)	1(50)
Ciprofloxacin	10	0(00)	2(100)	0(00)	0(00)	2(100)	0(00)
Erythromycin	30	0(00)	2(100)	0(00)	0(00)	2(100)	0(00)
Levofloxacin	20	0(00)	2(100)	0(00)	0(00)	2(100)	0(00)
Gentamycin	30	1(50)	1(50)	0(00)	0(00)	2(100)	0(00)
Cefuroxime	30	2(100)	0(00)	0(00)	1(50)	1(50)	0(00)
Rifampicin	20	1(50)	1(50)	0(00)	1(50)	1(50)	0(00)
Ceftazidime	30	1(50)	0(00)	1(50)	1(50)	1(50)	0(00)
Streptomycin	30	0(00)	1(50)	1(50)	0(00)	2(100)	0(00)
Azithromycin	10	0(00)	1(50)	1(50)	0(00)	2(100)	0(00)

Keys: R - Resistant, I - Intermediate, S - Susceptible, n(%) = number of isolates (percentage).

amoxicillin and gentamicin, while eight isolates were susceptible to ciprofloxacin and erythromycin. All nine isolates (100%) were susceptible to levofloxacin.

For *Staphylococcus* spp. (n = 7), four isolates were resistant to amoxicillin and ceftazidime, while all seven isolates (100%) were susceptible to ciprofloxacin and

levofloxacin.

Table 4 shows that both *Micrococcus* spp. isolates were completely susceptible (100%) to amoxicillin, ciprofloxacin, erythromycin, levofloxacin, gentamicin, and streptomycin. Both isolates were completely resistant (100%) to ceftazidime, cefuroxime, and rifampicin, while

one isolate was resistant to azithromycin.

The single *Enterococcus* sp. isolate was resistant to amoxicillin, gentamicin, cefuroxime, rifampicin, ceftazidime, streptomycin, and azithromycin, but was susceptible to ciprofloxacin, erythromycin, and levofloxacin.

Table 5 shows that both *Listeria* spp. isolates demonstrated complete resistance (100%) to cefuroxime and complete susceptibility (100%) to ciprofloxacin, erythromycin, and levofloxacin.

Both *Corynebacterium* spp. isolates (n = 2) were susceptible (100%) to ciprofloxacin, erythromycin, levofloxacin, gentamicin, streptomycin, and azithromycin. One isolate (50%) was resistant to each of amoxicillin, cefuroxime, rifampicin, and ceftazidime.

DISCUSSION

The total heterotrophic bacterial counts (THBC) of the sampled headphones ranged from 4.3×10^5 to 2.07×10^6 CFU/ml of swab suspension, while the total heterotrophic fungal counts (THFC) ranged from 1.2×10^4 to 4.1×10^4 SFU/ml of swab suspension. These counts indicate a substantial microbial load and highlight the potential role of frequently used personal devices, such as headphones, as fomites for microbial transmission.

The bacterial loads recorded in this study are comparable with findings from other frequently handled personal items and fomites. Onyemaechi et al. (2022) reported bacterial counts ranging from 10^5 to 10^6 CFU/ml on mobile phones used by students in Enugu, Nigeria. Similarly, Akinyemi et al. (2009) reported bacterial contamination of public telephones in Lagos, which was associated with continuous human contact. The bacterial loads observed in the present study may be associated with frequent handling and the warm and moist conditions around the ear, which may support microbial survival and proliferation.

The fungal counts obtained, ranging from 1.2×10^4 to 4.1×10^4 SFU/ml, also demonstrate that headphones can harbor fungi of potential public health significance. In a related study, Ugwu et al. (2020) reported fungal contamination of personal computer accessories frequently used by students, with counts ranging from 10^3 to 10^5 SFU/ml. These findings are comparable with those of the present study, although differences in the devices sampled and sampling procedures should be considered.

The higher bacterial counts compared with fungal counts observed in the present study are consistent with observations reported for other human-handled items (Olajide et al., 2015). Bacteria may predominate on frequently handled objects because of their efficient transfer from the skin, respiratory secretions, and the environment. However, differences in sampling procedures, culture conditions, and enumeration methods should be considered when comparing microbial loads

across studies.

The bacterial isolates recovered in this study included *Bacillus*, *Staphylococcus*, *Micrococcus*, *Corynebacterium*, *Enterococcus*, and *Listeria*. Similar genera have been reported from earphones and other skin-contact fomites. Ihemeje (2020), in a study of earphones used by students at Michael Okpara University, Umudike, Abia State, reported the isolation of *Staphylococcus aureus*, coagulase-negative *Staphylococci*, *Bacillus* spp., *Proteus*, *Escherichia coli*, and other environmental organisms.

The fungal isolates identified by colony morphology and microscopic examination included *Rhizopus*, *Aspergillus*, *Penicillium*, and *Candida*. Similar fungal genera have been reported in studies of otomycosis and contaminated personal devices. In Port Harcourt, Oparaodu et al. (2021) reported *Candida*, *Aspergillus*, and *Penicillium* among isolates from clinical cases of otomycosis. Similarly, Nwabuisi and Ologe (2001) reported *Aspergillus* and *Candida* among isolates obtained from patients with ear infections in Ilorin.

Fungi such as *Aspergillus* spp. are recognized as important causative agents of otomycosis, particularly in warm and humid climates. Risk factors include frequent ear cleaning, which may damage the ear canal, the use of foreign objects in the ear, poor hygiene, and environmental exposure (Oparaodu et al., 2021). However, the isolation of these fungi from headphones in the present study does not establish that the devices caused ear infections.

Among the 23 bacterial isolates identified in this study, *Bacillus* spp. were the most predominant, accounting for 9 (39.1%) isolates, followed by *Staphylococcus* spp. with 7 (30.4%). Other isolates included *Micrococcus* spp. 2 (8.7%), *Listeria* spp. 2 (8.7%), *Corynebacterium* spp. 2 (8.7%), and *Enterococcus* sp. 1 (4.3%).

The predominance of *Bacillus* spp. is consistent with the environmental distribution of members of this genus and their ability to survive under adverse environmental conditions. These characteristics may partly explain their recovery from frequently handled fomites (Igbeneghu and Lamikanra, 2014; Onwugamba et al., 2019).

Among the 11 fungal isolates, *Rhizopus* spp. and *Aspergillus* spp. each accounted for 4 (36.4%) isolates, followed by *Candida* spp. with 2 (18.2%) isolates and *Penicillium* spp. with 1 (9.1%) isolate. The occurrence of *Rhizopus* and *Aspergillus* is consistent with reports of environmental fungi isolated from earphones, fomites, and clinical ear samples (Ugwu et al., 2020). *Aspergillus niger* and *Aspergillus flavus*, in particular, have been implicated in otomycosis and allergic reactions (Adeleke et al., 2018). Their recovery from the sampled headphones indicates that these devices can become contaminated with fungi of potential clinical relevance.

The antifungal screening results showed varying responses to fluconazole, ketoconazole, and itraconazole. Among the fungal isolates, *Rhizopus* spp.

demonstrated substantial resistance to fluconazole and itraconazole, while comparatively greater inhibition was observed with ketoconazole at some concentrations. This observation is consistent with reports that *Rhizopus* species have reduced susceptibility to many azole antifungals (Chayakulkeeree et al., 2006). Similar findings have been reported regarding the limited effectiveness of some azoles against *Rhizopus* in otomycosis cases (Adeleke et al., 2018). However, because the present study employed a modified, non-standardized agar-well diffusion method, these observations should be regarded as experimental screening responses rather than definitive clinical antifungal resistance.

The *Candida* isolates demonstrated substantial resistance to fluconazole and itraconazole under several of the experimental conditions, while variable responses were observed with ketoconazole. This finding is consistent with reports of azole resistance among some *Candida* strains (Onwukeme et al., 2020). Although *Candida* may occur as part of the normal microbial flora, some species can cause opportunistic infections under favorable conditions. Nevertheless, the present study cannot establish a clinical risk of infection among headphone users.

The *Aspergillus* isolates showed high resistance to fluconazole under several experimental conditions, while comparatively better responses were observed with ketoconazole and itraconazole at some concentrations. Similar antifungal response patterns have been reported among *Aspergillus* isolates obtained from ear swabs and fomites, including lower activity of fluconazole and greater activity of itraconazole (Okunlola et al., 2017).

The single *Penicillium* isolate showed comparatively greater susceptibility to fluconazole and ketoconazole, with variable responses to itraconazole. This observation is consistent with previous reports that some *Penicillium* species may exhibit lower levels of resistance to antifungal agents than *Candida* and *Aspergillus* (Ezeonu and Ejikeme, 2019). However, interpretation is limited by the recovery of only one *Penicillium* isolate.

The antibacterial susceptibility patterns also demonstrated considerable variation among the bacterial isolates. *Bacillus* spp. showed complete (100%) susceptibility to levofloxacin, while resistance was observed to cefuroxime (66.7%), rifampicin (44.4%), and ceftazidime (77.8%). These findings can be compared with those of Al-Habibi et al. (2022), who reported multidrug resistance among *Bacillus* spp. isolated from hospital environments in Egypt.

Staphylococcus spp. showed 100% susceptibility to ciprofloxacin and levofloxacin, while resistance was observed to amoxicillin (57.1%) and ceftazidime (57.1%). This finding is comparable with the study of Udo et al. (2016), which reported high resistance of *Staphylococcus aureus* to beta-lactam antibiotics, including amoxicillin and cephalosporins, but greater susceptibility to

fluoroquinolones and macrolides. The resistance observed in the present study may reflect antimicrobial selection pressure; however, the small sample size does not permit conclusions about community-wide antibiotic use or misuse.

The two *Micrococcus* spp. isolates showed complete susceptibility to several antibiotics, including ciprofloxacin, erythromycin, levofloxacin, gentamicin, and streptomycin, but demonstrated complete resistance to cefuroxime and rifampicin. Similar findings were reported by Akinyemi et al. (2009) in Lagos, Nigeria, where *Micrococcus* species were generally susceptible to quinolones and aminoglycosides but exhibited variable resistance to beta-lactams.

The single *Enterococcus* sp. isolate exhibited resistance to amoxicillin, gentamicin, cefuroxime, rifampicin, ceftazidime, streptomycin, and azithromycin, while remaining susceptible to ciprofloxacin, erythromycin, and levofloxacin. Although this broad resistance profile is noteworthy, interpretation should be made cautiously because it was based on only one isolate. Similar resistant *Enterococcus* strains have been reported from clinical and environmental sources in Bayelsa State, Nigeria (Onanuga and Temedie, 2011).

The two *Listeria* spp. isolates showed complete susceptibility to ciprofloxacin, erythromycin, and levofloxacin but complete resistance to cefuroxime. These findings are comparable with reports that *Listeria* spp. may exhibit resistance to cephalosporins while remaining susceptible to some other antimicrobial agents (Morvan et al., 2010; Olonitola et al., 2015).

The *Corynebacterium* spp. isolates were completely susceptible to ciprofloxacin, erythromycin, levofloxacin, gentamicin, streptomycin, and azithromycin, while 50% resistance was observed to amoxicillin and cefuroxime. These findings are consistent with observations by Umoh et al. (2016), who reported susceptibility of *Corynebacterium* spp. to fluoroquinolones but variable resistance to beta-lactam antibiotics.

CONCLUSION

The findings of this study demonstrate that headphones used by students at Rivers State University can harbor diverse bacterial and fungal microorganisms and may therefore serve as potential fomites. The bacterial isolates included organisms commonly associated with the skin and environment, while the fungal isolates included genera that have been reported in otomycosis and other opportunistic infections. The warm and humid environmental conditions of Port Harcourt may favor microbial persistence; however, this study did not directly assess environmental factors or establish a causal relationship between headphone contamination and ear infection.

The antimicrobial screening results also demonstrated variable responses among the bacterial and fungal isolates. However, the antifungal findings were obtained using a modified, non-standardized agar-well diffusion method and should therefore be interpreted as experimental screening observations rather than definitive clinical resistance profiles. Similarly, the small number of bacterial isolates limits the extent to which the observed resistance patterns can be generalized.

RECOMMENDATIONS

1. Students should be educated on the importance of regularly cleaning and disinfecting headphones, particularly ear cushions and other components that come into contact with the skin.
2. Headphones with porous ear cushions may retain moisture and potentially support microbial persistence; therefore, non-porous materials or removable and washable cushions may be preferable.
3. Sharing of headphones should be discouraged, particularly when adequate cleaning and disinfection cannot be performed between users.
4. Subsequent studies should investigate the relationship between microbial load and user-related factors, including frequency of headphone use, cleaning practices, sharing frequency, storage conditions, material type, and personal hygiene.
5. Future studies should include larger sample sizes, appropriate control groups, standardized surface-area sampling, and standardized antimicrobial susceptibility methods to improve the reliability and generalizability of the findings.

LIMITATIONS OF THE STUDY

The study had several limitations. The small sample size of ten headphones and the absence of a control group limit the statistical strength and generalizability of the findings. The sampled surface area was not standardized, making it impossible to express microbial contamination per unit surface area. In addition, user-related factors such as cleaning practices, frequency of sharing, duration of use, and storage conditions were not systematically measured and could not be evaluated as potential determinants of microbial contamination.

Furthermore, fungal susceptibility was assessed using a modified agar-well diffusion method rather than a standardized clinical susceptibility-testing procedure. Therefore, the antifungal responses should be interpreted as assay-specific screening findings and not as definitive clinical resistance or susceptibility profiles. Finally, the study relied on culture-based methods and therefore describes only the cultivable microorganisms recovered under the conditions employed.

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