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Determination and Antibiogram of Bacteria Flora Associated with Grass and Feathers Used for Self- Ear Cleaning in Port Harcourt

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ABSTRACT

Self-ear cleaning is the insertion of objects into the ear canal. It is a widespread practice that has the potential to compromise ear integrity as a natural self-cleansing mechanism and the risk of possible microbial infections. This study aimed to determine the bacterial flora associated with grass and feathers used for self-ear cleaning. A total of 10 samples (5 Grass and 5 Feathers) were gotten from two different locations in Port Harcourt Local Government Area; Mile3 Market and Rivers State University. The samples were packed into ten different sterile, transparent zip-lock bags. They were properly labeled and transported to the Microbiology Laboratory of the Rivers State University for proper analysis. The result showed that the total heterotrophic bacteria count (THBC) of grass and feather ranged from $2.4x10^{-5}$ to 6.5x10-5 and $1.3x10^{-6}$ to $2.3x10^{-6,}$ respectively. A total of 31 bacterial isolates belonging to five (5) genera comprising both Gram-negative and Gram-positive organisms were identified. The bacterial flora associated with grass showed a predominance of Bacillus spp. (38.8%), Escherichia coli (33.3%), Pseudomonas sp. (11.1%), and lower occurrence of Staphylococcus devriesei (5.6%), Staphylococcus arlettae (5.6%), and Micrococcus sp (5.6%). The predominant organisms isolated from feathers were Escherichia coli (23.1%), Pseudomonas spp. (23.1%), Bacillus sp(15.4%), while various Staphylococcus species: S. pasteuri (7.7%), S. lutrae(7.7%), S. jettensis(7.7%), S. aureus (7.7%), and Micrococcus species(7.7%) had lower occurrence. The antibiotic susceptibility results showed that Gram-negative isolates such as E. coli showed susceptibility to ofloxacin, ciprofloxacin, and trimethoprim while being resistant to ceftazidime, augmentin, and ceporex. Gram-positive isolates like Bacillus sp. showed resistance to ceftriaxone and ceftazidime while being susceptible to levofloxacin and gentamicin. Grass and feathers harbor a lot of microorganisms, which may pose lots of health challenges to humans due to bacterial infection. Therefore, it is recommended to avoid using grass and feathers for self-ear cleaning, as they are non- sterile and can lead to damage of the external auditory canal. Seeking medical help should also be encouraged, and any pain, discharge, or hearing loss should prompt medical evaluation rather than using grass and feathers.

Keywords: *Ear cleaning, Grass, Feathers, Bacteria, Antibiogram.*

Introduction

Self-ear cleaning is the insertion of objects into the ear canal. It is a widespread practice that has the potential to compromise its integrity as a natural self-cleansing mechanism and the risk factor for possible injuries [20].

In developing regions, the morbidity and mortality associated with ear disease and injuries remain a significant but neglected public health concern [19]. The external auditory meatus can clean itself, which is made possible by the cleansing function of cerumen, a naturally occurring substance that cleans, protects, and lubricates the external auditory canal. It is usually unnecessary to clean the ear canal as excessive cleaning increases humidity and softens the ear canal lining, which can result in infection and irritation of the ear. [19].

Cerumen, with its content of lysosomes, glycoproteins, immunoglobulin, lipids, and trace elements, has a bactericidal action that plays a significant role in maintaining the local host defense mechanism in the ear [19]. It has a high acidic pH (about 4 to 5), which is unfavorable for organisms and helps reduce the risk of infection in the auditory canal. An unprofessional attempt to clean the ear canal or habitual wax removal is a potential risk for ear-related symptoms and injuries, including pain, earache, bleeding, tympanic membrane perforations, and weakening of the external auditory canals' local defense against bacterial and fungal infections. The risk is even greater when this is done as a blind procedure without direct inspection of the ear canal using objects not designed to remove wax and foreign bodies in the ear, such as cotton buds and loose tip cotton swabs, feathers, grass, sticks, and a variety of other objects.

The use of grass and feathers for ear cleaning is a long-standing traditional practice still observed in various rural communities. Throughout history, different cultures have developed various methods for cleaning the ears, ranging from natural remedies to modern medical techniques.

In many rural communities, traditional ear cleaning methods have been passed down from generation to generation. Certain types of grass, particularly those with barbed or brittle seed heads, increase the risk of foreign body retention in the ear canal, leading to discomfort and medical complications. [14].
Health professionals are trained to clean ears by safer means if medically required (7). Earwax lubricates, cleans, and protects the external auditory canal [9]. Self-ear cleaning rids the ear of this wax and potentially leads to ear infections, trauma, and perforation of the tympanic membrane as objects are inserted blindly into the ear canal [4].
Additionally, the use of unsterilized plant material introduces bacteria, which can cause ear infections. [20]. In many traditional settings, people use natural materials for hygiene due to accessibility and long-standing beliefs about their effectiveness. However, the associated hazards outweigh safer alternatives. Medical professionals strongly discourage inserting foreign objects into the ear, as improper cleaning techniques can result in tympanic membrane damage, chronic inflammation, and even permanent hearing loss [14].

Materials and Methods

Study Area

This study was carried out in Rivers State University and Mile 3 market in Port Harcourt Local Government Area of Rivers State. The coordinates are presented in Table 1

Table 1: Geographical Coordinates

Sample Location	Coordinates
EN, Rivers State University, Port Harcourt	Lat 4.79616° Lon 6.978777°
BL 1, Rivers State University, Port Harcourt	Lat 4.976318° Lon 6.978772°
SC, Rivers state University Port Harcourt	Lat 4.796767° Lon 6.97873°
BL 1, Rivers State University port Harcourt	Lat 4.796905° Lon 6.978617°
BL 2, Rivers State University port Harcourt.	Lat 4.697772° Lon 6.979053°
Mile 3 Market	Lat 4.804337° Lon 6.993703°
Mile 3 Market	Lat 4.804313° Lon 6.993773°
Mile 3 Market	Lat 4.804324° Lon 6.993719°
Mile 3 Market	Lat 4.804409° Lon 6.993652°
Mile 3 Market	Lat 4.804223° Lon 6.993676°

KEY: *EN= Engineering, BL1=Biology lab 1, SC=Science class, BL2=Biology lab 2*

Sample Collection and Preparation

A total of 10 samples (5 Grass and 5 Feather) were gotten from two different locations in Port Harcourt Local Government Area; Mile3 Market and Rivers State University. The samples were packed into ten different sterile transparent zip-lock bags, properly labeled, and transported to the Microbiology Laboratory of the Rivers State University for proper analysis. 1g of the different samples (5 grass and 5 feathers) was weighed and soaked in peptone water for two hours, and 1ml was diluted into 9ml of Normal saline (diluent), to resuscitate the organism.

Serial Dilution

1g of the different samples (5 grass and 5 feathers) was weighed and soaked in peptone water for two hours, and 1ml was diluted into 9ml of Normal saline (diluent), to give a solution of 10^{-1}. An aliquot of the solution was then pipette from 10^{-1} and transferred to 10^{-2} up till 10^{-4} dilutions. An aliquot of the solution was plated into the already prepared Nutrient agar (NA), Eosin Methylene Blue agar (EMB), and MacConkey agar (MCA) plates. It was spread evenly using a bent glass rod sterilized by dipping it into ethanol and passing it over a flame. The plates were inverted and put into an incubator at 37°C for 24 hours.

Microbiological Assessment of Grass and Feathers Inoculation and Isolation of Microorganisms

1g of 10 different samples (5 grass and 5 feathers) was weighed and put into sterile test tubes containing 9ml of normal saline (diluent) and thoroughly swirled. The suspension was diluted using a tenfold serial dilution. Aliquots of the appropriate dilutions were plated and spread in the already prepared media (Nutrient agar, MacConkey agar, and Eosin Methylene Blue agar). The NA, MCA, and EMB were Inverted and incubated at 37°C for 24hours. After isolation, the number of discrete colonies was counted in the form of colony-forming units per gram (Cfu/g). The visible counts were obtained, and the bacteria were used to enumerate the total visible counts of the colonies in colony-forming units per gram. (Cfu/g).

Characterization and identification of pure culture

Discrete colonies were enumerated and sub-cultured into freshly prepared Nutrient agar, MacConkey agar, and Eosin Methylene Blue agar by streak plate technique to obtain pure isolates/colonies. This was done on a sterile working bench. The wire loop was flamed into red-hot and allowed to cool in the air, after which a loopful of the isolates was taken and streaked on an already prepared plate for bacterial growth. The sub-cultured media plates were incubated at 37°C for 24hours.

Characterization and Identification of Bacterial Isolates.

Cultural characterization was observed on the plates. The colonial characteristics were grouped under forms/shape, elevation, margin, surface texture, size, color, and opacity.
A physical analysis was done on the isolates to determine their morphology. Microscopic examination was carried out by subjecting them to Gram staining to determine their staining reaction as gram-negative or gram-positive organisms.

Results

Bacteria count

The result shows the total heterotrophic bacteria count (THBC) of grass and feathers. The data obtained shows the total heterotrophic counts of bacteria colonies ranging from the lowest to the highest count, $2.4x10^{-5}$ - $6.5x10^{-5}$ for Grass and $1.3x10^{-6}$ - $2.3x10^{-6}$ for feather (Table 2).

Table 2: Total Heterotrophic Bacteria Count of Grass and Feather Used for Ear Cleaning

Samples	Total Heterotrophic Bacteria Count (THBC)
G1	$2.4x10^{5}$
G2	$3.4x10^{5}$
G3	$2.6x10^{5}$
G4	$3.7x10^{5}$
G5	$6.5x10^{5}$
F1	$1.3x10^{5}$
F2	$1.7x10^{5}$
F3	$1.4x10^{6}$
F4	$2.3x10^{6}$
F5	$1.6x10^{6}$

KEY: *G1-G5=Grass. F1-F5=feathers*

Biochemical test results isolated from Grass and Feathers

Table 3 shows different biochemical test used to characterize bacteria species. The organisms isolated were *Escherichia coli. Pseudomonas sp., Micrococcus sp., Bacillus sp,* and *Staphylococcus* species.

Table 3: Biochemical Test Results of Bacteria Isolates from Grass and Feathers Used for Ear Cleaning

Isolate code	Gram Rxn	CAT	OXI	INDO	MOT	CIT	MR	VP	GLU	MAN	SUC	LAC	ORGANISM
G3	-ve rods	+ve	-ve	+ve	+ve	-ve	+ve	-ve	AG	AG	AG	AG	*Escherichia coli*
G 1	+ve cocci	+ve	-ve	-ve	-ve	+ve	+ve	+ve	A	A	A	A	*Staphylococcus devriesei*
F1	+ve cocci	+ve	-ve	-ve	-ve	-ve	+ve	+ve	A	A	A	A	*Staphylococcus Pasteur*
F1	-ve rods	+ve	-ve	-ve	+ve	+ve	-ve	-ve	N	N	N	N	*Pseudomonas* sp.
G1	+ve rods	+ve	-ve	+ve	+ve	+ve	+ve	+ve	A	AG	A	A	*Bacillus* sp.
F1	+ve cocci	+ve	-ve	-ve	+ve	-ve	+ve	+ve	AG	A	AG	A	*Staphylococcus lutrae*
F1	-ve rods	+ve	-ve	-ve	+ve	-ve	+ve	-ve	AG	A	A	A	*Escherichia coli*
G2	-ve rods	+ve	-ve	-ve	+ve	-ve	+ve	+ve	N	N	N	N	*Pseudomonas* sp.
F3	+ve rods	+ve	+ve	-ve	+ve	+ve	-ve	+ve	A	A	A	N	*Bacillus* sp.
F3	-ve rods	+ve	+ve	-ve	+ve	+ve	-ve	-ve	N	N	N	N	*Pseudomonas* sp.
F3	+ve cocci	+ve	-ve	-ve	-ve	+ve	-ve	-ve	A	A	N	N	*Micrococcus* sp.
G2	-ve rods	+ve	-ve	-ve	+ve	-ve	+ve	+ve	A	A	A	A	*Escherichia coli*
G2	-ve rods	+ve	-ve	-ve	+ve	+ve	+ve	-ve	A	A	A	A	*Escherichia coli*
F2	+ve cocci	+ve	-ve	-ve	-ve	-ve	+ve	+ve	A	N	A	A	*Staphylococcus jettensis*
F2	-ve rods	+ve	+ve	-ve	+ve	+ve	-ve	-ve	N	N	N	N	*Pseudomonas* sp.
G1	+ve cocci	+ve	-ve	-ve	-ve	+ve	-ve	-ve	A	A	N	N	*Micrococcus* sp.
G1	-ve rods	+ve	-ve	+ve	+ve	-ve	+ve	+ve	AG	AG	AG	AG	*Escherichia coli*
G2	-ve rods	+ve	-ve	-ve	+ve	-ve	+ve	+ve	A	A	A	A	*Escherichia coli*
G3	+ve rods	+ve	+ve	-ve	+ve	+ve	-ve	+ve	A	A	A	N	*Bacillus* sp.
G3	+ve rods	+ve	+ve	-ve	+ve	+ve	+ve	+ve	A	A	A	N	*Bacillus* sp.
F5	-ve rods	+ve	+ve	+ve	+ve	-ve	-ve	-ve	AG	AG	AG	AG	*Escherichia coli*
G2	+ve rods	+ve	+ve	-ve	+ve	+ve	-ve	-ve	A	A	A	N	*Bacillus* sp.
G4	+ve rods	+ve	+ve	-ve	+ve	+ve	-ve	+ve	A	A	A	N	*Bacillus* sp.
G4	-ve rods	+ve	+ve	-ve	+ve	-ve	-ve	-ve	N	N	N	N	*Pseudomonas* sp.
G5	+ve rods	+ve	+ve	-ve	-ve	+ve	+ve	-ve	A	N	A	N	*Bacillus* sp.
G5	+ve cocci	+ve	-ve	-ve	-ve	+ve	-ve	+ve	A	N	AG	N	*Staphylococcus arlettae*
F1	-ve rods	+ve	-ve	-ve	+ve	-ve	+ve	-ve	A	A	A	A	*Escherichia coli*
F2	-ve rods	+ve	-ve	+ve	+ve	-ve	-ve	-ve	AG	AG	AG	AG	*Escherichia coli*
G1	+ve rods	+ve	+ve	-ve	+ve	+ve	+ve	+ve	A	A	A	N	*Bacillus* sp.
G3	+ve rods	+ve	+ve	-ve	+ve	-ve	+ve	+ve	A	A	A	N	*Bacillus* sp.
F1	+ve cocci	+ve	-ve	-ve	-ve	-ve	+ve	+ve	A	A	A	A	*Staphylococcus aureus*

***KEY**: G1-G5=Grass, F1-F5=Feather, Gram Rxn=Gram Reaction, CAT=Catalase; OXI=Oxidase; INDO=Indole, MOT=Motility test, CIT=Citrate, MR=Methyl Red, VP=Vogue Proskauer, GLU=Glucose, MAN=Mannitol, SUC=Sucrose, LAC=Lactose*

Percentage Occurrence of Bacteria Isolated from Grass Samples

The percentage occurrence of bacteria isolated from grass is presented in Figure 1. *Bacillus* sp had the highest occurrence (38.8%), *Escherichia coli* (33.3%), *Pseudomonas* sp (11.1%), *Staphylococcus devrisei, Staphylococcus arlette, and Micrococcus* sp. had the lowest occurrence (5.6%), respectively.

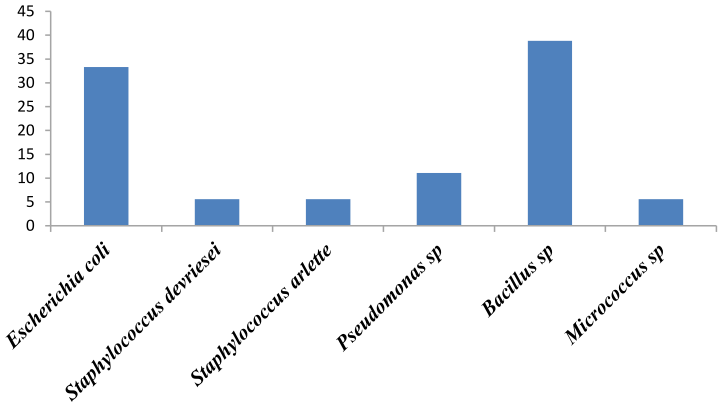

Figure 1: Percentage Occurrence of Bacteria Isolated from Grass Samples Used for Ear Cleaning

Percentage Occurrence of Bacteria Isolated from Feather Samples

The percentage occurrence of bacteria isolated from feather samples is presented in Figure 2. *Escherichia coli* and *Pseudomonas* had the highest occurrences (23.1% respectively), and *Staphylococcus pasteuri, Staphylococcus lutrae, Staphylococcus jettensis, Staphylococcusand, Micrococcus* sp were the lowest occurrences (7.7% respectively).

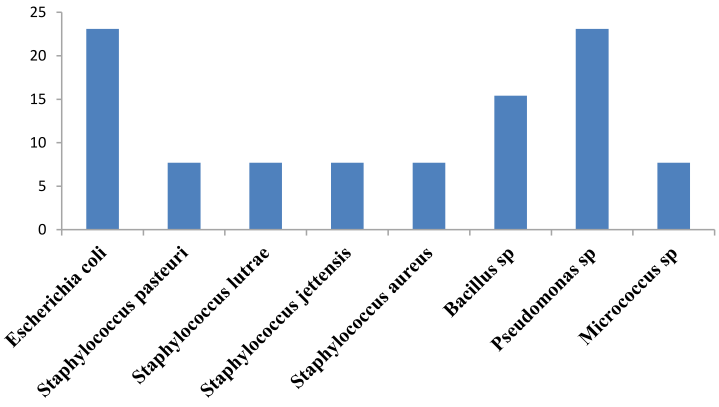

Figure 2: Percentage Occurrence of Bacteria Isolated from Feather Samples Used for Ear Cleaning

Antibiotic Susceptibility Pattern of Gram-positive Bacteria Isolated from Grass and Feather Samples

Tables 4-6 shows antibiotics susceptibility pattern of both Gram-positive and Gram-negative bacteria isolated from Grass and Feather. Results show that*Staphylococcus species* were resistant to Ceftriaxone, Ceftazidine, Rifampicin, Ciprofloxacin, and susceptible to Gentamicin, Levofloxacin, and Amoxicillin.*E. coli* showed susceptibility to ofloxacin, ciprofloxacin, and trimethoprim while being resistant to ceftazidime, augmentin, and ceporex.

Table 4: Antibiotic Susceptibility Pattern of Staphylococcus Species from Grass and Feather Samples

	Staphylococcus devriesei(1)			*Staphylococcus lutrae*(1)		
Antibiotic(conc.ug)	**R N%**	**I N%**	**S N%**	**R N%**	**I N%**	**S N%**
Levofloxacin(5ug)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)
Gentamicin(10ug)	0(0.00)	0(0.00)	1(100)	1(100)	0(0.00)	0(0.00)
Ceftriaxone(30ug)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)
Rifampicin(5ug)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)
Ceftazidine(30ug)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)
Streptomycin(25ug)	0(0.00)	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)
Azithromycin(5ug)	0(0.00)	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)
Amoxicillin(25ug)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)
Ciprofloxacin(5ug)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)
Erythromycin(15ug)	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)	0(0.00)
	Staphylococcus pasteuri(1)			*Staphylococcus jettensis*(1)		
Antibiotics(conc.ug)	**R N%**	**I N%**	**S N%**	**R N%**	**I N%**	**S N%**
Levofloxacin(5ug)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)
Gentamicin(10ug)	0(0.00)	0(0.00)	1(100)	1(100)	0(0.00)	0(0.00)
Ceftriaxone(30ug)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)
Rifampicin(5ug)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)
Ceftazidine(30ug)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)
Streptomycin(25ug)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)
Azithromycin(5ug)	1(100)	0(0.00)	0(0.00)	0(0.00)	1(100)	0(0.00)
Amoxicillin(25ug)	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)	0(0.00)
Ciprofloxacin(5ug)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)
Erythromycin(15ug)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)
	Staphylococcus arlettae(1)			*Staphylococcus aureus* (1)		
Antibiotics(conc.ug)	**R N%**	**I N%**	**S N%**	**R N%**	**I N%**	**S N%**
Levofloxacin(5ug)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100)
Gentamicin(10ug)	1(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100)
Ceftriaxone(30ug)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)
Rifampicin(5ug)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)
Ceftazidine(30ug)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)
Streptomycin(25ug)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)
Azithromycin(5ug)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)
Amoxicillin(25ug)	0(0.00)	0(0.00)	1(100)	1(100)	0(0.00)	0(0.00)
Ciprofloxacin(5ug)	1(100)	0(0.00)	0(0.00)	0(0.00)	1(100)	0(0.00)
Erythromycin(15ug)	1(100)	0(0.00)	0(0.00)	0(0.00)	1(100)	0(0.00)

Antibiotic Susceptibility Pattern of *Bacillus* sp and *Micrococcus* sp. Bacteria Isolated from Grass and Feather Samples.

The antibiotic susceptibility pattern of *Bacillus* sp. and M*icrococcus* sp. is as presented in Table 5. Results show that *Bacillus* sp. was highly resistant to Ceftriaxone, Ceftazidine, Erythromycin, and Susceptible to levofloxacin and Gentamicin. *Micrococcus* sp. was resistant to levofloxacin, ceftriaxone, Streptomycin, Azithromycin, and Amoxicillin.

Table 5: Antibiotics Susceptibility Pattern of Bacillus sp. and Micrococcus sp. from Grass and Feather Samples

	Bacillus sp. *(9)*			*Micrococcus* sp. *(2)*		
Antibiotics(conc.ug)	**R N%**	**I N%**	**S N%**	**R N%**	**I N%**	**S N%**
Levofloxacin(5ug)	0(0.00)	0(0.00)	9(100)	1(50.0)	0(0.00)	1(50.0)
Gentamicin(10ug)	0(0.00)	1(11.1)	8(88.9)	0(0.00)	0(0.00)	2(100)
Ceftriaxone(30ug)	8(88.9)	0(0.00)	1(11.1)	1(50.0)	1(50.0)	0(0.00)
Rifampicin(5ug)	0(0.00)	4(44.4)	5(55.6)	0(0.00)	1(50.0)	1(50.0)
Ceftazidine(30ug)	7(77.7)	0(0.00)	1(11.1)	1(50.0)	0(0.00)	1(50.0)
Streptomycin(25ug)	0(0.00)	2(22.2)	7(77.7)	2(100)	0(0.00)	0(0.00)
Azithromycin(5ug)	4(44.4)	1(11.1)	4(44.4)	1(50.0)	0(0.00)	1(50.0)
Amoxicillin(25ug)	3(33.3)	3(33.3)	3(33.3)	1(50.0)	1(50.0)	0(0.00)
Ciprofloxacin(5ug)	0(0.00)	6(66.6)	3(33.3)	0(0.00)	1(50.0)	1(50.0)
Erythromycin(15ug)	6(66.6)	3(33.3)	0(0.00)	2(100)	0(0.00)	0(0.00)

Antibiotics Susceptibility Pattern of Gram-negative Bacteria Isolated from Grass and Feather Samples.

The result shows the antibiotic susceptibility pattern of *Escherichia coli* and Pseudomonas sp. (Table 6). *Pseudomonas* sp. was resistant to Ceftazidine, Augmentin, Ceporex and susceptible to Trimethoprim, Streptomycin, and Ciprofloxacin. *Escherichia coli* was resistant to Ofloxacin, Ciprofloxacin, and Trimethoprim.

Table 6: Antibiotic susceptibility pattern of Pseudomonas sp. and Escherichia coli

Antibiotics(conc.ug)	*Pseudomonas sp*(5)			*Escherichia coli* (9)		
	R N%	I N%	S N%	R N%	I N%	S N%
Ceftriaxone(30ug)	3(60.0)	2(40.0)	0(0.00)	5(55.6)	2(22.2)	2(22.2)
Ofloxacin (5ug)	2(40.0)	0(0.00)	3(60.0)	0(0.00)	0(0.00)	9(100)
Augmentin	5(100)	0(0.00)	0(0.00)	8(88.9)	0(0.00)	1(11.1)
Pefloxacin(5ug)	3(60.0)	0(0.00)	2(40.0)	7(77.8)	0(0.00)	2(22.2)
Ceftazidine (30ug)	5(100)	0(0.00)	0(0.00)	9(100)	0(0.00)	0(0.00)
Gentamicin (10ug)	2(40.0)	0(0.00)	3(60.0)	4(44.4)	0(0.00)	5(55.6)
Ciprofloxacin (5ug)	2(40.0)	0(0.00)	4(80.0)	1(11.1)	1(11.1)	7(77.8)
Ceporex (30ug)	5(100)	0(0.00)	0(0.00)	6(66.7)	1(11.1)	2(22.2)
Trimethoprim (5ug)	0(0.00)	1(20.0)	4(80.0)	3(33.3)	0(0.00)	6(66.7)
Streptomycin(25ug)	1(20.0)	0(0.00)	4(80.0)	3(33.3)	1(11.1)	5(55.6)

Discussion

The bacterial load recorded in this study shows that both grass and feathers used for ear cleaning carry substantial microbial populations, with feathers consistently harboring higher counts. The grass samples had THBC values ranging from 2.4×10^{-5} to 6.5×10^{-5} CFU/ml, while feather samples ranged between 1.3×10^{-6} and 2.3×10^{-6} CFU/ml. These findings align with the observations of [11], who reported that feathers commonly harbor diverse bacterial communities, including *Bacillus* spp. and *Staphylococci*, due to their keratinous composition and capacity to trap organic matter. Similarly, [2] identified feathers among the objects frequently used for ear cleaning in Nigeria and emphasized their potential role in harboring bacteria that predispose users to otitis externa. The agreement between these studies and the present findings highlights the potential risk feathers pose as vehicles for bacterial transfer into the ear canal.

There are, however, consistencies and discrepancies when compared with studies on microbial contamination of plant-based implements. [17] found high bacterial counts on wooden toothpicks used for oral hygiene, with isolates including *Staphylococcus aureus*, *E. coli*, and *Bacillus* spp. The similarity in microbial flora and counts from the present study, suggests that porous organic materials, whether plant-based like grass and wood or animal-based like feathers, provide niches that support bacterial colonization. [3] also demonstrated widespread contamination of African herbal preparations by *E. coli*, *Staphylococcus aureus*, and Enterobacteriaceae, further corroborating the tendency of natural substrates to support microbial growth. The agreement here suggests that the microbial burden is not unique to feathers or grass but is a broader phenomenon associated with natural organic materials.

The present study shows higher counts in feather samples compared to grass, which differs from similar findings. For example, [10] reported discomfort and complications associated with grass stalks more frequently than with feathers in their Ghanaian cohort. This apparent disagreement may be due to variations in sample handling, environmental exposure, or the nature of the grass species studied. Grass stalks, depending on their growth environment, may carry higher levels of soil-associated organisms. In contrast, feathers, especially those derived from domestic birds, often harbor both skin- and fecal-associated bacteria [23]. Differences in microbial ecology between environments and sources of materials thus likely explain the variation in reported loads.

The predominance of organisms such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* in ear infections, as reported by [14, 15], agrees with the expectation that some of the bacterial flora identified in feather and grass samples could act as opportunistic pathogens once introduced into the ear canal.

The consistency between clinical isolates and potential environmental sources reemphasizes the significance of these findings to otitis externa. Nonetheless, the higher mean THBC of feather samples in this study compared with grass, contrasts with reports where plant-derived objects (sticks and grass) were more implicated in ear trauma and subsequent infections [2]. This difference may be due to the fact thatbecause trauma frequency, rather than microbial load alone, often dictates which objects are more commonly linked to ear infections.

The diversity of bacteria isolated from this study indicates that both grass and feathers are potential reservoirs of bacteria, including potentially opportunistic and pathogenic species of public health concern.

Escherichia coli was one of the most frequently isolated bacteria, occurring in both grass and feather samples. *E. coli* is a common gut commensal and also an opportunistic pathogen that can cause ear infections, urinary tract infections, and gastrointestinal diseases [13]. Similar findings were reported by [22], who isolated *E. coli* from locally used objects for ear cleaning, emphasizing its persistence in environments exposed to handling and contamination. The agreement in findings emphasizes that grass and feathers may act as vehicles for enteric pathogens, which increases risk, when inserted into the ear canal.

Staphylococcus species were also prominent among the isolates, including *S. aureus* and coagulase-negative staphylococci such as *S. devriesei, S. pasteuri, S. lutrae, S. jettensis,* and *S. arlettae*. The isolation of *S. aureus* is particularly concerning, as it is a well-known etiological agent of otitis externa and skin infections [8]. In a similar study, [16] reported a high prevalence of *Staphylococcus* spp. from ear swabs of patients with otitis, suggesting that contaminated materials such as feathers may contribute to the spread of these organisms into the ear canal. The agreement across studies signifies the potential risk of self-ear cleaning with non-sterile materials.

The bacterial flora associated with grass used for ear cleaning showed a predominance of *Bacillus* species (38.8%), followed by *Escherichia coli* (33.3%), *Pseudomonas species* (11.1%), and lower occurrences of *Staphylococcus devriesei* (5.6%), *Staphylococcus arlettae* (5.6%), and *Micrococcus* species (5.6%). The distribution suggests that grasses, being environmental materials, harbor both environmental bacteria (e.g., *Bacillus, Micrococcus*) and potentially pathogenic microorganisms (e.g., *E. coli, Pseudomonas, Staphylococcus*). The bacterial flora associated with feathers used for ear cleaning showed a more diverse but evenly distributed microbial profile compared to grass. The predominant organisms were *Escherichia coli* (23.1%) and *Pseudomonas* species (23.1%), followed by *Bacillus* species (15.4%), while various *Staphylococcus* species (*S. pasteuri, S. lutrae, S. jettensis, S. aureus*) and *Micrococcus* species were each represented at 7.7%.

When compared with grass isolates (Figures 1 and 2), feathers harbored a greater diversity of *Staphylococcus* species, while grass was dominated by *Bacillus* spp. The higher prevalence of *E. coli* and *Pseudomonas* in feathers may reflect greater contact with animal sources and moisture retention in feathers, both of which support the growth of these bacteria. These findings are in agreement with studies on poultry feathers that revealed diverse bacterial colonization, including *E. coli, Pseudomonas, and Staphylococcus* [18].

The antibiotic susceptibility profiles of the isolates from this study showed that Gram-negative isolates, such as *E. coli* and *Pseudomonas* spp. Showed resistance to several β-lactam antibiotics but retained varying degrees of susceptibility to fluoroquinolones such as ciprofloxacin and ofloxacin. This result agrees with the findings from similar studies where Gram-negative organisms isolated from otitis media and environmental samples commonly exhibited multidrug resistance but remained susceptible to fluoroquinolones [1] [5]. Such resistance can be attributed to indiscriminate antibiotic use [21]. The similarity between these studies suggests that ear-cleaning materials may serve as silent reservoirs of resistant Gram-negative organisms, predisposing individuals to difficult-to-treat ear infections.

Interestingly, *Bacillus* spp., though traditionally regarded as environmental contaminants, exhibited resistance to multiple first-line antibiotics. This observation is in line with [6], who reported resistant *Bacillus* strains from fomites and hospital environments in Lagos. Such findings challenge the notion that *Bacillus* species are always benign, as resistant strains can cause opportunistic infections under favorable conditions.

Conclusion and Recommendation

In synopsis, the present study can posit that feathers and grass used for ear cleaning can act as both reservoirs and vehicles of bacterial transmission and consequently, has have the potential to cause ear infections in individuals that who use them, as well as, pose significant public health risks.

The findings from the present study signify that both sample materials used for ear cleaning, harbour a variety of bacterial species, some of which have been implicated in ear infections and other opportunistic diseases. This emphasizes the idea regarding the norm of self-ear cleaning with non-sterile materials, which may introduce potentially pathogenic microorganisms directly into the ear canal.

The susceptibility result confirms that bacteria associated with grass and feathers used for ear cleaning not only emphasizes the resistance trends globally, but also serve as potential sources of resistant infections. The use of such materials exposes users to both the risk of direct inoculation of pathogenic bacteria into the ear canal and the potential transfer of multidrug-resistant strains.

It is therefore advised, that the use of grass and feathers for self-ear cleaning should be discouraged, as they are non- sterile and can lead to damage of the external auditory canal. Alternatives like ear irrigation or micro- suction with the recommendation of an ear doctor, should be used.

Rural dwellers should also be enlightened, as some rural dwellers use these materials due to poor knowledge, and public health education should focus on risks encountered when using grass and feathers.

Further studies on those that still practice the traditional ear-cleaning method should be encouraged to assess microbial risk.

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