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INCIDENCE AND ANTIBIOTICS SUSCEPTIBILITY PATTERN OF BACTERIA OF EAR ORIGIN

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ABSTRACT: This study is aimed at investigating both the Gram-positive and the Gram-negative bacteria of ear swabs and their antibiotic susceptibility pattern. Ear samples of one hundred (100) subjects using sterile swab sticks were collected. Sixty-seven (67) out of 100 samples collected yielded growth when incubated at 37 ºC for 24 h. Microbiological tests were carried out to characterize the bacterial isolates. Kirby-Baur disk diffusion method was employed for antibiotic sensitivity testing. Results showed 67 isolates; *Staphylococcus aureus* 39 (58.2%): *Streptococcus pyogenes* (17.9%) and *Pseudomonas aeruginosa* (23.9%). The result obtained also showed incidence rates of Gram-positive and Gram-negative organisms to be 76.1% and 23.9%, respectively. The isolates demonstrated varying susceptibility patterns. Gram-negative organisms showed the highest susceptibility to Augumetin (93.8%) and Chloranphenicol (87.5%), while the Gram-positive organisms showed the highest susceptibility to Ceftriaxone (80.4%) and Ciprofloxacin (84.3%). Both Gram-positive and Gram-negative organisms showed the lowest susceptibility to Amoxicillin at 52.9% and 56.3%, respectively. It revealed that the bacterial isolates present had low susceptibility to generally available drugs; this leaves health care professionals with little options when this normal flora and opportunistic pathogens cause infection.

INTRODUCTION: Otitis media, is a condition associated with the inflammation of the middle ear cleft, it is a universal problem. Clinically, it is grouped into as acute, sub-acute, and chronic, depending on time. The incidence rate acute otitis media is 10.85% while that of chronic suppurative otitis media (CSOM) incidence rate is 4.76% [1].

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The spread of Infection spread from middle-ear to other essential structures such as nerve, mastoid, facial labyrinth, meninges, lateral sinus, and brain resulting in a mastoid abscess, deafness, facial nerve paralysis, meningitis, lateral sinus thrombosis, and intracranial abscess [2].

Untreated or poorly treated OM owing to either erroneous diagnosis or unsuitable use of antibiotics causes to purulent otitis, frequently with perforation and complications including the persistence of middle ear effusion which demands the placing of the drainage tube and regularly leading to hearing impairment, mastoiditis, abscess meningitis, COM, brain and sepsis [3, 4].

It can also cause impaired speech and poor school performance language development, and impaired social interaction [5]. An ear infection is a common problem for both children and adults, but the magnitude is different in different countries. In children's ear anatomy, Eustachian tube is known to be shorter, more horizontal having more flaccid cartilage that can easily harm its opening, and consequently, an ear infection is a major health problem of them, especially in those with poor socioeconomic status [6].

CSOM shares several properties of diseases such as Chagas and Trachoma, including unreasonably affecting persons living in poverty leading to noteworthy morbidity, could be amenable to public health intervention, and is neglected by research [7]. The majority of the microbiological studies had exposed the most common bacteria connected to ear diseases are *Pseudomonas*, *Proteus* spp, *Staphylococcus*, and *Klebsiella* [2]. Aim to determine the frequency of bacterial isolates within the ear and their drug susceptibility patterns against commonly used topical antibiotics as well as other antibiotics from patients who gave ear discharge samples at a tertiary care teaching hospital.

MATERIALS AND METHODS:

Material:

Reagents and Media: Mac Conkey agar, nutrient agar, Mannitol salt agar, Mullerr-hinton agar, peptone water. Kovac's reagent, phenol red, 3% hydrogen peroxide, alcohol, lead acetate paper.

Apparatus: Autoclave, incubator, test tubes, wire-loop, conical flask, beakers cotton wool, aluminum, analytical balance, microscope, glass slide, petri-dishes.

Ethical Consideration: Before samples were collected, information regarding the study was explained to the community individuals after approval was sought from the head of the Institution. Oral consent for participation in the study was obtained.

Sample Collection: This study was conducted from June 2016 to February 2017 on one hundred (100) ear isolates of students. The study was undertaken in Delta State University Abraka in Delta State, with an aim to study the bacterial flora and their sensitivities to a series of antibiotics in-patients with ear infections. Ear samples of patients were collected and then sent to the microbiology lab for microbiological studies in the Department of Pharmaceutical Microbiology were. The collected samples were immediately cultured on blood agar, chocolate agar, and Mac Conkey agar. Standard bacteriological procedures were used to isolate and identify aerobic bacteria [8]. All cases showing growth on culture media after 72 h of inoculation were subjected to antibiotic sensitivity testing by modified Kirby -Bauer disk diffusion method, and the interpretation of results was done by using standard guidelines [8].

Antibiotics Susceptibility Testing: The antibiotic sensitivity testing of the isolates was evaluated using the Kirby-Bauer disk diffusion method. 0.2 ml of the standardized test was added into the surface of well-set agar in a petri-dish, and this was uniformly spread.

The antibiotics disc, which includes ciprofloxacin, Co-trimoxazole, Ceftriaxone, gentamicin, ampiclox Sparfloxacin erythromycin Streptomycin, Amoxicillin, and Chloramphenicol was carefully removed from the cartridges and placed firmly on the agar plate with the aid of pair sterile forceps. The agar plates were then incubated at 37 ºC for 24 h. The zones of inhibition were measured and recorded [8].

RESULTS: Ear swabs were collected from hundred (100) students of Delta State University Abraka. Bacteria isolates were identified by cultural, morphological, and biochemical methods/characteristics, which include *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. On inoculation, sixty-seven (67) yielded growth, and they were identified and analyzed. Out of the 67 strains identified, 51 (76%) were Gram positives (Coci spp and 16 (24%) were Gram-negatives. From the fifty-one (51) isolates of the Cocci species showed in **Table 1**, *Streptococcus pyrogen* represents [4] (23.5%) and *Staphylococcus aureus* 39 (76.5%).

From the results, *Staphylococcus aureus* alone accounts for 58% of the total isolates obtained. The sixteen (16) strains of Gram-negatives organisms showed in **Table 2**, were all identified to be *Pseudomonas aeruginosa*.

TABLE 1: IDENTIFICATION OF GRAM POSITIVE BACTERIA ISOLATES

Isolates	Shape	GS	MSA	Catalase	Coagulase	Motility	Orgianism
1	*Cocci*	+ve	-	-	-	-	*S. pyogenes*
2	*Cocci*	+ve	-	-	-	-	*S. pyogenes*
3	*Cocci*	+ve	-	-	-	-	*S. pyogenes*
4	*Cocci*	+ve	-	-	-	-	*S. pyogenes*
5	*Cocci*	+ve	-	-	-	-	*S. pyogenes*
6	*Cocci*	+ve	-	-	-	-	*S. pyogenes*
7	*Cocci*	+ve	-	-	-	-	*S. pyogenes*
8	*Cocci*	+ve	-	-	-	-	*S. pyogenes*
9	*Cocci*	+ve	-	-	-	-	*S. pyogenes*
10	*Cocci*	+ve	-	-	-	-	*S. pyogenes*
11	*Cocci*	+ve	-	-	-	-	*S. pyogenes*
12	*Cocci*	+ve	-	-	-	-	*S. pyogenes*
13	*Cocci*	+ve	+	+	+	-	*S. aureus*
14	*Cocci*	+ve	+	+	+	-	*S. aureus*
15	*Cocci*	+ve	+	+	+	-	*S. aureus*
16	*Cocci*	+ve	+	+	+	-	*S. aureus*
17	*Cocci*	+ve	+	+	+	-	*S. aureus*
18	*Cocci*	+ve	+	+	+	-	*S. aureus*
19	*Cocci*	+ve	+	+	+	-	*S. aureus*
20	*Cocci*	+ve	+	+	+	-	*S. aureus*
21	*Cocci*	+ve	+	+	+	-	*S. aureus*
22	*Cocci*	+ve	+	+	+	-	*S. aureus*
23	*Cocci*	+ve	+	+	+	-	*S. aureus*
24	*Cocci*	+ve	+	+	+	-	*S. aureus*
25	*Cocci*	+ve	+	+	+	-	*S. aureus*
26	*Cocci*	+ve	+	+	+	-	*S. aureus*
27	*Cocci*	+ve	+	+	+	-	*S. aureus*
28	*Cocci*	+ve	+	+	+	-	*S. aureus*
29	*Cocci*	+ve	+	+	+	-	*S. aureus*
30	*Cocci*	+ve	+	+	+	-	*S. aureus*
31	*Cocci*	+ve	+	+	+	-	*S. aureus*
32	*Cocci*	+ve	+	+	+	-	*S. aureus*
33	*Cocci*	+ve	+	+	+	-	*S. aureus*
34	*Cocci*	+ve	+	+	+	-	*S. aureus*
35	*Cocci*	+ve	+	+	+	-	*S. aureus*
36	*Cocci*	+ve	+	+	+	-	*S. aureus*
37	*Cocci*	+ve	+	+	+	-	*S. aureus*
38	*Cocci*	+ve	+	+	+	-	*S. aureus*
39	*Cocci*	+ve	+	+	+	-	*S. aureus*
40	*Cocci*	+ve	+	+	+	-	*S. aureus*
41	*Cocci*	+ve	+	+	+	-	*S. aureus*
42	*Cocci*	+ve	+	+	+	-	*S. aureus*
43	*Cocci*	+ve	+	+	+	-	*S. aureus*
44	*Cocci*	+ve	+	+	+	-	*S. aureus*
45	*Cocci*	+ve	+	+	+	-	*S. aureus*
46	*Cocci*	+ve	+	+	+	-	*S. aureus*
47	*Cocci*	+ve	+	+	+	-	*S. aureus*
48	*Cocci*	+ve	+	+	+	-	*S. aureus*
49	*Cocci*	+ve	+	+	+	-	*S. aureus*
50	*Cocci*	+ve	+	+	+	-	*S. aureus*
51	*Cocci*	+ve	+	+	+	-	*S. aureus*

Key: GS = Gram Stain, MSA = Mannitol salt agar, Gram-positive, - = Gram negative

Gram Positive; *Staphylococcus aureus* isolates are the most prevalent bacteria and showed the following susceptibility to the antibiotics used: 80.4% ciprofloxacin; 69.2% (gentamicin); 59% (Ampiclox); 38/5% (amoxicillin); 74.4% (Ceftriaxone); 69.2% (Cotrimoxazole); 66.7% (Streptomycin); and 76.9% (Erythromycin). *Staphylococcus pyogenes* showed 100% susceptibility to ciprofloxacin and cefriaxone; 83.3% (gentamicin); 66.7% (ampiclox); 75% (amoxicillin); 91.7% (septrin); 83.3% (streptomycin).

TABLE 2: IDENTIFICATION OF GRAM-NEGATIVE BACTERIA ISOLATES

Isolates	Shape	GS	MCA	Indole	H_2S	Catalase	Motility	Organism
52	Rods	-ve	+	-	-	+	+	*Pseudomonas spp*
53	Rods	-ve	+	-	-	+	+	*Pseudomonas spp*
54	Rods	-ve	+	-	-	+	+	*Pseudomonas spp*
55	Rods	-ve	+	-	-	+	+	*Pseudomonas spp*
56	Rods	-ve	+	-	-	+	+	*Pseudomonas spp*
57	Rods	-ve	+	-	-	+	+	*Pseudomonas spp*
58	Rods	-ve	+	-	-	+	+	*Pseudomonas spp*
59	Rods	-ve	+	-	-	+	+	*Pseudomonas spp*
60	Rods	-ve	+	-	-	+	+	*Pseudomonas spp*
61	Rods	-ve	+	-	-	+	+	*Pseudomonas spp*
62	Rods	-ve	+	-	-	+	+	*Pseudomonas spp*
63	Rods	-ve	+	-	-	+	+	*Pseudomonas spp*
64	Rods	-ve	+	-	-	+	+	*Pseudomonas spp*
65	Rods	-ve	+	-	-	+	+	*Pseudomonas spp*
66	Rods	-ve	+	-	-	+	+	*Pseudomonas spp*
67	Rods	-ve	+	-	-	+	+	*Pseudomonas spp*

The mean susceptibility pattern of cocci species isolate against the antibiotics showed: ciprofloxacin (84.3%), streptomycin (76.6%), erythromycin (76.5%), ceftriaxone (72.5%), Cotrimoxazole (74.5%), ampiclox (60.8%), amoxicillin (52.9%). The highest susceptibility of the Gram-positive isolates was to Ciprofloxacin (84.3%) and Ceftriaxone (80.4%) while they showed the lowest susceptibility to amoxicillin (52.9%) followed by ampiclox (60.8%).

TABLE 3: SUSCEPTIBILITY OF GRAM POSITIVE BACTERIA SENSITIVE TO COMMONLY USED ANTIBIOTICS

Organism	GN	APX	AM	Cef	CPX	S	SEP	E
Staphylococcus aureus (39)	27 (69.2)	23 (59)	15 (38.5)	29 (74.4)	31 (80.4)	26 (66.7)	27 (67.7)	30 (76.9)
Streptococcus pyogenes (12)	10 (83.3)	8 (66.7)	9 (75)	12 (100)	12 (100)	10 (83.3)	11 (91.7)	9 (75)

Key: S = Streptomycin, Cot = Cotrimoxazole, CH = Chloramphenicol, E = Erythromycin, CPX = Ciprofloxacin, AM = Amoxicillin, Cef = Ceftriaxone GN = Gentamicin, APX = Ampiclox

For the Gram-negative isolates as shown in **Table 4**, which are all *Pseudomonas aeruginosa* had antibiotic susceptibility of 62.5% (streptomycin), 68.8% (chloramphenicol), 81.3% (Sparfloxacin), 75% (ciprofloxacin), 56.3% (amoxicillin), 93.8% (augmentin) and 75% (gentamicin. The highest susceptibility of the Gram-negative isolates was to augmentin (93.8%) and chloramphenicol (87.5%) while they showed the lowest susceptibility to amoxicillin (56.3%) followed by streptomycin (62.5%).

TABLE 4: SUSCEPTIBILITY PATTERN OF GRAM-NEGATIVE BACTERIA TO COMMONLY USED ANTIBIOTICS

Organism	AU	SP	CPX	CH	GN	S	AM	COT
Pseudomonas aeruginosa (16)	14(87.5%)	12(75%)	12(75%)	13(81.3%)	12(75%)	11(68.8%)	10(62.5%)	11(68.8%)

Key: S = Streptomycin, COT = Cotrimoxazole, CH = Chloramphenicol, SP = Sparfloxacin, CPX = Ciprofloxacin, AM = Amoxicillin, AU = Augmentin, GN = Gentamicin

DISCUSSION: The Human ear is made up of three major parts; outer, middle, and inner ear. The outer ear comprises of the pinna and the auditory ear canal to the tympanic membrane. The inner ear, also known as the bony labyrinth, is a fluid-filled compartment that surrounds the membranous labyrinth, which encloses both the cochlea and the vestibules apparatus [9]. This study dealt with the incidence and sensitivity patterns of the bacteria isolated from the ear of subjects at Delta State University Abraka. Out of the 67 strains identified, 51 (76%) were Gram positives (*cocci* spp and 16 (24%) were Gram-negatives. From the fifty-one (51) isolates of the *cocci* species showed in **Table 1** and **2**.

Streptococcus pyrogen represents 12 (23.5%) and *Staphylococcus aureus* 39 (76.5%). The sixteen (16) isolates of Gram-negatives organisms showed in **Table 3**, were all identified to be *Pseudomonas aeruginosa* [10]. From the data above, *Staphylococcus aureus* accounts for 58% of the entire bacterial

isolates obtained. The incidence rate of the number of various bacteria identified from samples collected from students after analysis were Staphylococcus species and Streptococcus species with thirty-nine (39) and twelve (12) number of isolates respectively and *Pseudomonas* spp. with sixteen (16) numbers of isolates, as seen in **Table 1** and **2**.

The isolation rate of bacteria from the ear in this study is 67%, and Staphylococcus specie is the most prevalent, similar result was obtained by 11 and reports of some other researchers from Mekelle and Addis Ababa [4]. Unlike our findings, Proteus spp. followed by *S. aureus* and *Pseudomonas* spp. were the predominant isolates reported by other investigators from different parts of Ethiopia [12] and fairly different patterns were reported from elsewhere [13] with *P. aeruginosa* as the major isolate followed by *S. aureus* and *Proteus* spp. The possible reasons for such variation in the bacterial profile might be attributed to the difference in climatic and geographic variation of the study sites, unlike the report of [10] who reported that *Pseudomonas aeruginosa* (34.21%) was the most common aerobic isolate in ear discharge which was very closely followed by *Staphylococcus aureus* (27.63%).

Panchal *et al.,* reported that aural swabs collected from patients of chronic suppurative otitis media showed *Pseudomonas* in 36% cases, followed by *S. aureus* in 30% of cases. *Pseudomonas aeruginosa* and *Staphylococcus aureus* account for 61.84% of total bacterial isolates in cases of CSOM. This lower prevalence is due to such factors as a patient not being screened for infection, education level of the subjects, and sample size variation. The bacteria isolated from the sample include *Staphylococcus aureus* (58.2%), *Streptococcus pyogenes* (17.9%) and *Pseudomonas aeruginosa* (23.2%). These values are higher for *Staphylococcus aureus,* which shows is the most prevalent at all. These values are also similar to the range of frequencies reported in other countries, such as Cameroon [14]. The high level of colonization of the ear by *Staphylococcus aureus,* as seen in this study, has equally been reported by other researchers [15]. The frequent isolation of *Pseudomonas aeruginosa* indicates that individuals are at high risk of infection due to poor hygiene conditions [17]. *Pseudomonas aeruginosa* and *Staphylococcus aureus* account for 61.84% of total bacterial isolates in cases of ear infection [16].

Antibiotic susceptibility carried out on isolates from this study showed that both Gram-negative and the Gram-positive organisms showed the highest susceptibility to augmentin and ciprofloxacin, respectively, and lowest susceptibility to amoxicillin [15]. This result is similar to the one obtained in related study [16]. *Streptococcus pyogene* was 100% sensitive to Ceftriaxone, and ciprofloxacin (80.4%) and erythromycin (76.9%) recorded the highest sensitivity than other antibiotics (gentamycin, streptomycin, septrin, amipclox, ceftriaxone, and amoxicillin). The antibiotic array of resistant strains resulting from continued and irrational use of these older drugs over the years [17].

However, a very low rate of susceptibility to ampiclox was recorded, and antibiotic susceptibility test carried out Gram-positive organisms from the study revealed relatively low susceptibility to penicillins. This might be due to antibiotic misuse or abuse [18]. The susceptibility pattern of the gram-negative organisms, as shown in **Table 6** revealed low susceptible to amoxicillin (56.3%) and Streptomycin (62.5%).

Pseudomonas aeruginosa had high sensitivity to amoxicillin (87.5%) gentamicin, (75%) ciprofloxacin (92.3%), and High fluoroquinolones antibacterial activity against Pseudomonas isolates were reported by others, although resistant strains of Pseudomonas isolates to fluoroquinolones were detected in other studies [19]. According to Arshi *et al.,* 2019, *Staphylococcus aureus* was sensitive to gentamicin (90.47%), ciprofloxacin (90.47%), and ofloxacin (71.42%).

CONCLUSION: The present study has revealed that despite the ear being healthy, is still houses a variety or microbes because of its exposure to the outside environment. It does not necessarily mean the ear has to be infections are caused by bacteria, and many of them are considered to be normal flora of the ear. The result of this present study revealed that *Staphylococcus aureus* is the most common bacterial isolate from the ear. The choice of antibiotic depends on the pattern exhibited locally,

and the selection of antibiotics varies based on the specimen and isolate under consideration. From the result gotten, in-ear infection, it is more advisable to use cephalosporins than penicillins.

RECOMMENDATION: I recommend that those with ear infection should avoid uninformed self-medication with antibiotics in order to prevent them from building resistance to such drugs, they should ensure to see a physician for drug advice.

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