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Original Article

Implications of Biofilm-Producing Organisms Among Bacteria Isolated From Ear-, Nose-, and Throat-Infected Patients

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Abstract

Background: Bacteria resistant to antimicrobial agents have remained a major challenge in public health, and bacterial-producing biofilm is one of the main causes of antibiotic resistance, especially in upper respiratory tract infections (URTI). This study aimed at determining the antibiotic resistance pattern and formation of biofilms in bacteria causing ear, nose, and throat (ENT) infections in our study population.

Methods: One hundred and fifty samples, including ear (n=87), nasal discharge (22), throat swab (8), and surgical sample (33) (aspirate and tissue), were screened and analyzed using the culture technique, direct microscopy, and bacteria identification with an API 20E strip. The antibiotic susceptibility testing of the isolates was performed with Kirby-Bauer's disk diffusion techniques and interpreted based on the Clinical and Laboratory Standard Institute guidelines. The biofilm-producing organisms (BPOs) were determined by using the tube method technique.

Results: A total of 192 isolates were recovered (60% gram-positive and 40% gram-negative bacteria). Eighty-three (43.2%) of recovered isolates were multidrug-resistant (MDR) to antibiotics tested, and 60 (75%) isolates from MDR isolates were BPOs.

Conclusion: Biofilm-producing bacteria have higher tendencies to dominate in body-infected tissues other than the discharges being produced; therefore, tissue biopsy for culture and sensitivity should be considered more appropriate where visible, especially when confronted with hard-to-treat infections in ENT clinical settings.

Keywords: Ear, nose, and throat infection; Antibiotic resistance; Biofilm-producing organism

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Introduction

Antimicrobial resistance (AMR) is a significant public health threat, as admitted by the World Health Organization (WHO) (1). The extent of this AMR and the selection of multidrug-resistant (MDR) pathogens have been reported to be a result of non-compliance with proper infection control methods, unfounded use of antibiotics, availability of antibiotics without a prescription, and counterfeit products of dubious quality (2). Upper respiratory tract infections (URTI) are commonly treated with antibiotics based on the known sensitivity patterns of the common causative pathogens encountered in specific regions (3). Antimicrobial agents remain the backbone of infectious disease treatment; however, the unjustifiable uses of antibiotics in many countries have evolved into the emergence of MDR microorganisms (4). Infections become chronic, incalcitrant, or present with complications when treated with antibiotics that are not responsive to infective agents. The abuse of antimicrobial agents is well known to create pressure on the selection of appropriate drugs and resultantly increase the capability of microbes to restrain from being attacked (5). Antibiotic resistance leads to higher medical expenses, prolonged hospital stays, and increased mortality rates (2). Studies have reported an alarming increase in the occurrence of antibiotic-resistant bacteria that include beta-lactam-resistant strains of common pathogens as well as macrolides and fluoroquinolone-resistant strains isolated from URTIs (6,7).

An important element that contributes to AMR is biofilm production by bacteria. The formation of biofilm has been reported as one of the factors causing antibiotic

resistance, particularly in URTIs. Bacterial biofilms are naturally resistant to antibiotics due to the fact that some antibiotics are unable to reach the depths of the biofilm; some cells in biofilms grow slowly or not at all, likely due to nutrient limitation, and certain cells in the biofilms may adopt a unique and safeguarded biofilm phenotype (8).

Studies have identified bacterial biofilm as the main cause of antibiotic resistance in URTIs (9). Biofilm can form on moist biotic and abiotic surfaces, making them common for infection of the ear, nose, and throat (ENT). Bacterial biofilms are known to be "influencers of infections", especially in patients suffering from rhinosinusitis and otitis media as a result of their propensity to form biofilms in sinuses and adenoid tissues (9–13). Most bacteria (more than 99%) produce biofilms, which can lead to dangerous, incurable illnesses. The bacteria in biofilms interact with each other through molecular mechanisms that enable some cells to resist antibiotics and host immune defenses, hence increasing the likelihood that ENT infections would recur or persist (13).

It has been estimated that at least 25% of cases of chronic rhinosinusitis are caused by biofilm formation. (14) Many microorganisms such as *Streptococcus* spp., *Staphylococcus aureus*, *Corynebacterium argentoratense*, and *Micrococcus luteus* present in the respiratory tract have been reported to easily produce biofilms. *S. aureus* strains have been identified intracellularly and in the sub-mucosa of adult patients with chronic rhinosinusitis undergoing endoscopic sinus surgery (15). Microorganisms such as *S. aureus*, *Haemophilus influenza*, *Pseudomonas aeruginosa*, and fungus have been isolated and identified as bacterial biofilms from chronic rhinosinusitis (9).

Despite the knowledge of bacteria as the major etiological agents of URTI and the roles of antibiotic resistance and formation of biofilms in the pathogenesis of URTI, local studies have not focused on biofilm formation potentials in bacteria causing ENT infections in West Africa. Therefore, this study seeks to establish the antibiotic sensitivity pattern and formation of biofilms in bacteria causing ENT infections in our study population and to determine the contribution of biofilm formation to MDR agents and the most effective choice of empiric antibiotics.

Materials and Methods

Sampling Population/Ethical Clearance

Participants with a clinical diagnosis of ENT infections attending the Otorhinolaryngology (ENT) Clinic in Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Nigeria during the study period formed the study population. Ethical clearance (with protocol number ERC/2018/06/14) was duly obtained from the Ethical and Research Committee of the OAUTHC. In addition, informed consent was obtained from the patient or guardian of the patient as appropriate.

Sample Collection

ENT samples in the form of discharges, aspirates, and infected tissues were collected at the Otorhinolaryngology Clinic and Main Theatre of OAUTHC. Each sample was collected aseptically using sterile swab sticks or sterile bottles as appropriate by a medical doctor who has been pre-trained on the study protocol in the otorhinolaryngology clinic and operating theatre. The samples were collected into freshly prepared sterile transport media (thioglycolate media), properly labelled (with study number, date, gender, age, and time), and immediately transported for bacteriological analysis at the Microbiology Department of Obafemi Awolowo University, Ile-Ife, Nigeria.

Bacteria Isolation and Identification

The sample collected in the transport media was incubated over 24 hours at 37 ºC. The incubated culture was then inoculated separately on sterile blood agar, nutrient agar, MacConkey agar, and Mannitol salt agar (Lab M Ltd., UK) by the streak plate method for discrete colonies and incubated at 37 °C for 24 hours. The organisms were purified by successive subculturing on a nutrient agar plate. The isolates were identified by morphological and physiological characteristics according to Bergey's Manual of Determinative (16). Furthermore, the isolates were identified by using the MICROBACT™ identification kits 24E (Oxoid Ltd., UK) for Gram-negative, and the *STPY* gene was used to identify *S. aureus* using the polymerase chain reaction method.

Antibiotic Susceptibility Test and Biofilm Formation

The antibiotic susceptibility profile of the isolates was determined on Mueller-Hinton agar (MHA; Lab M Ltd., UK) according to Kirby-Bauer's disc diffusion technique (17). The antibiotic discs, including single (Oxoid Ltd., Basingstoke, Hampshire, England) and combined (Abtek Biological Ltd., UK) discs of varying and specific concentrations, were employed and aseptically placed on the inoculated MHA plate with sterile forceps. The antibiotic discs were properly placed on MHA plates, seeded with standardized (10^6 CFU/mL of 0.5 McFarland Standard) inoculum, and the plates were incubated at 37 °C for 18–24 hours, after which the diameter of zones of inhibition was compared with the Clinical and Laboratory Standard Institute (18) chart of interpretative zones as sensitive, resistance, and intermediate resistance. The isolates were described as resistant to multiple antibiotics when they were resistant to ≥ 3 separate classes of the tested antibiotics. The qualitative method for the biofilm formation of the isolates was performed using the tube method. A loop full of test organisms was inoculated in 10 mL of nutrient broth (Lab M Ltd., UK) with 1% glucose in test tubes. After incubation, the tubes were decanted and washed with the use of the phosphate buffer saline (pH = 7.3). The tubes were dried and then stained with crystal violet (0.1%). Deionized water was used to wash off excess stains, and the tubes were dried in an inverted

position. The tube method was scored in line with the results from the control strains. As depicted in Figure 1, biofilm formation was considered positive when a visible film lined the wall and the bottom of the tube. No biofilm was produced when the tube was clear, implying that the wall and bottom of the tube were not lined by any visible film (19).

Statistical Analysis

The data collected for analysis included patient sociodemographic details, previous exposure to antibiotics in current ENT disease, nature of the specimen, nature of isolates, antibiotic resistance, and biofilm production. Statistical Product and Service Solutions Statistics (version 22) was utilized to perform statistical analysis with the level of statistical significance set at $P \leq 0.05$.

Results

One hundred and fifty samples, including ear (n = 87), nasal (n = 22) throat (n = 8), and ENT surgical aspirates and tissues (n = 33), were obtained from 150 patients [77 (51.3%) females and 73 (48.7%) males] diagnosed with various ENT infections. The age range of 0–5 years had the highest population in this study, followed by age ≥ 46, while 41–45 years had the lowest population. Table 1 provides a summary of the collected samples.

As outlined in Tables 2 and 3, a total of 15 different bacterial species were identified from 192 bacteria isolated from 150 samples [110 (73.3%) mono-bacterial were cultured from collected samples, and poly-bacterial culture was found in 40 (26.70%) samples]. These species were *Proteus* spp., *P. aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Citrobacter freundii*, *Enterobacter cloacae*, *Serratia* spp., *Salmonella* spp., *Acinetobacter baumannii*, *Staphylococcus* spp., *Bacillus* spp., *Corynebacterium* spp., *Streptococcus* spp., *Micrococcus* spp., and *Enterococcus* spp. Antibiotic susceptibility showed meropenem, vancomycin, and ofloxacin as the most effective antibiotics, while the isolates are more resistant to commonly used empirical antibiotics such as augmentin, cotrimoxazole, ceftazidime, cefuroxime, gentamycin, erythromycin, chloramphenicol, and ampicillin.

Based on the results (Table 4), 192 bacteria were recovered from collected samples, 113 isolates were isolated from ear infections, and 51 (45.1%) were multiple antibiotic resistant (MAR). In addition, 46 isolates were recovered from nose infection, and 20 (43.5%) were MAR. Twenty-three isolates were recovered from the throat, while 12 (52.2%) were MAR.

The biofilm formation of multiple antibiotic-resistant bacterial isolates cultured from ENT infections is represented in Tables 5, 6, and Figure 2. Out of 113 isolates that were isolated from ear infections, 51 (45.1%) were MAR, and 36 (70.6%) were biofilm-producing organisms (BPOs). Further, from nose infection, there was a total of 46 isolates, including 20 (43.5%) MAR and 16 (80%) BPO. A total number of 23 isolates were recovered from the throat, including 13 (52.2%) MAR and 10 (83.3%) BPO. Of all collected samples (from both tissue and discharge samples), all the tissue samples that were multiple antibiotic resistant for gram-positive bacteria were 91.7% biofilm producers, and 100% of gram-negative bacteria were BPOs, while 71% and 61.8% were discharge samples and biofilm producers for both gram-positive and gram-negative, respectively.

Discussion

In this study, ear infections had the highest prevalence, followed by nasal and throat infections, which corroborates the findings of studies performed by Sharma et al (20) and Otoghile et al (21) in Guwahati, India and River State,

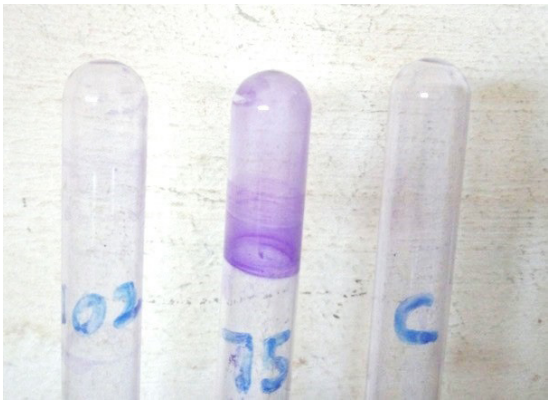

Figure 1. An Image of Biofilm-Producing Bacteria Recovered From ENT Infections. *Note*. ENT: Ear, nose, and throat

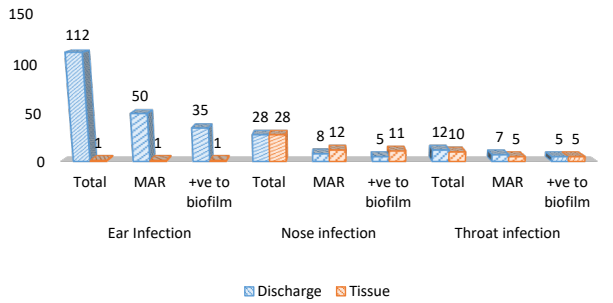

Figure 2. The Frequency of Biofilm-Producing Bacteria Isolated From ENT Infections Related to Their Site. *Note*. ENT: Ear, nose, and throat

Table 1. Nature of Samples Collected From Their Site of Infection

Nature of the Sample	Site of Infection			
	Ear infection n = 88 (58.7%)	Nose Infection n = 46 (30.6%)	Throat infection n = 16 (30.6%)	Total n = 150 (100)
Discharge	87 (98.9)	22 (47.8)	8 (50.0)	117 (78)
Tissue	1 (1.1)	24 (52.2)	8 (50.0)	33 (22)

Nigeria, respectively. Studies have identified different bacterial as aetiology agents causing ENT infections (22-26). In this study, the main isolated bacteria were *S. aureus* (21.4%), *Proteus* spp. (15.1%), *Staphylococcus* spp. (10.9%), *Corynebacterium* spp., and *P. aeruginosa* (10.4%), respectively. Similar to our reports, other investigators such as Obiajuru and Chukuezi (22) and Ahmad et al (24) implicated *S. aureus* as the most prevalent organism in Imo and Kano States in Nigeria, respectively. However, El-Mahmood et al (6) and Al-Badaii et al (26) reported *Streptococcus pyogenes*, *S. aureus*, *Klebsiella pneumoniae*, and *P. aeruginosa* as the commonest bacteria causing infections in Yola and Dhamar Governorate, Yemen, respectively. Generally, in this study, Gram-negative bacteria were most sensitive to meropenem and ofloxacin, while the Gram-positive bacteria were most sensitive to both vancomycin, meropenem, and ofloxacin, suggesting that these drugs may be adopted as empiric antibiotics. Heidari et al (27) also found *P. aeruginosa* as the main culprit, which also demonstrated high resistance to ciprofloxacin and amikacin. The observations showed that the isolates are highly resistant to beta-lactam, which is the most current empirical antibiotic used in our locality. The findings of previous usage of antibiotics commonly found among patients with drug resistance are not unexpected given the poor regulation of drug dispensing and usage in the Nigerian environment. The finding of this study underscores the need for periodic reviews of empiric antibiotics in our environment.

In this study, almost all the isolates, which are multiple antibiotic resistant, were positive for biofilm production, especially isolates from tissue cultures. *S. aureus* and *P. aeruginosa* isolates were able to produce biofilms in all the sites of ENT infection. The biofilm-producing bacteria have a high tendency to be more dominant in tissue samples compared to discharge samples, as found in this study. Their ability to stick to a surface or tissue, freely flow in the bloodstream, evade the immune system of the host, and resist the effect of appropriate drugs has been reported to play a major role in the persistence of bacterial infections. This ability has enabled them to avoid the possibility of being washed away through water flow or bloodstream, to oppose many bodily factors that can hamper the formation and effect of biofilm, and to tolerate any harsh environmental conditions (28-30). Therefore, this can enhance their potential to resist antibiotics. In other words, biofilm generation can make antibiotics lose their ability to fight bacterial infections by protecting bacteria strains from antibiotic agents and immune system cells, especially those who would have ordinarily been overpowered by appropriate medications

Table 2. Antibiotic Susceptibility Pattern of Gram-positive Bacteria Cultured From Ear, Nose, and Throat Infection Samples

Antibiotic (µg)	No. of Isolates	Number of Isolate Occurrence (%)		
		Susceptibility	Intermediate	Resistance
VAN	115	115 (100)	0 (0)	0 (0)
MEM	115	114 (99)	1 (1)	0 (0)
OFL	115	108 (94)	6 (5)	1 (1)
AUG	115	62 (54)	0 (0)	53 (46)
AMP	115	62 (54)	2 (1.7)	51 (44)
CRX	115	53 (46)	11 (10)	51 (44)
COT	115	65 (56.5)	2 (1.7)	48 (41.7)
GEN	115	64 (56)	22 (19)	29 (25)
CAZ	115	42 (36.5)	16 (13.9)	57 (49.6)
TET	115	49 (42.6)	26 (22.6)	40 (34.8)
ERY	115	46 (40)	42 (36.5)	27 (23.5)

Note. CAZ: Ceftazidime (30 µg); CRX: Cefuroxime (30 µg); OFL: Ofloxacin (5 µg); AUG: Augmentin (30 µg); NIT: Nitrofurantoin (300 µg); CPR: Ciprofloxacin (5 µg); GEN: Gentamycin (10 µg); CXM: Cefixime (5 µg); CHL: Chloramphenicol (30 µg); TET: Tetracycline (30 µg); MEM: Meropenem (10 µg); FOX: Cefoxitin (30 µg); COT: Cotrimoxazole (25 µg); CTX: Cefotaxime (30 µg); CTR: Ceftriaxone (30 µg); AMK: Amikacin (30 µg); ERY: Erythromycin; VAN: Vancomycin; AMP: Ampicillin (10 µg).

Table 3. Antibiotic Susceptibility Pattern of Gram-negative Bacterial Cultured From Ear, Nose, and Throat Infection Samples

Antibiotics (µg)	No. of Isolates	Number of Isolate Occurrence (%)		
		Susceptibility	Intermediate	Resistance
MEM	77	77 (100)	0 (0)	0 (0)
OFL	77	69 (89.6)	1 (1.3)	7 (9.1)
CXM	77	49 (63.6)	8 (10.4)	20 (26)
NIT	77	48 (62.3)	11 (14.3)	18 (23.4)
CPR	77	64 (83.1)	6 (7.8)	7 (9.1)
FOX	77	46 (59.7)	11 (14.3)	20 (26)
GEN	77	48 (62.3)	9 (11.7)	20 (26)
CHL	77	41 (53.2)	14 (18.2)	22 (28.6)
CTR	77	38 (49.4)	25 (32.5)	14 (18.2)
AMK	77	33 (42.9)	11 (14.3)	23 (29.9)
COT	77	33 (42.9)	8 (10.4)	36 (46.8)
CTX	77	28 (36.4)	30 (39)	19 (24.7)
CAZ	77	26 (33.8)	26 (33.8)	25 (32.5)
CRX	77	23 (29.9)	19 (24.7)	35 (45.5)
AUG	77	22 (28.6)	26 (33.8)	29 (37.7)
TET	77	26 (33.8)	9 (11.7)	42 (54.5)

Table 4. Frequency of Multidrug Gram-negative and Gram-Positive Isolates Cultured ENT Infection Samples

Site of infection	Ear Infection		Nose Infection		Throat Infection	
Nature of the sample	Number of Isolates	MAR	Number of Isolates	MAR	Number of Isolates	MAR
Discharge	112	50	28	8	13	7
Tissue	1	1	28	12	10	5
Overall total	113	51 (45.1%)	56	20 (35.7%)	23	12 (52.2%)

Note. ENT: Ear, nose, and throat; MAR: Multiple antibiotics resistant.

Table 5. Biofilm Formation of Multiple Antibiotics Resistant Isolate Cultured From Ear, Nose, and Throat Infection Samples

Gram-Negative Bacteria Isolate	Ear Infection			Nose Infection			Throat Infection		
	No. of Isolates (n=56)	No. of MAR (n=31)	No. of+ve BF (n=20)	No. of isolates (n=16)	No. of MAR (n=8)	No. of+ve BF (n=6)	No. of isolates (n=5)	No. of MAR (n=1)	No. of+ve BF (n=1)
Proteus spp.	22 (39.2%)	9 (29%)	8 (40%)	5 (31.2%)	1 (12.5%)	1 (16.7)	2 (40%)	0	0
Pseudomonas spp.	15 (26.8%)	13 (41.9%)	7 (35%)	4 (25%)	3 (37.5%)	2 (33.3)	1 (20%)	1 (100%)	1 (100%)
Escherichia coil	4 (7.1%)	3 (9.7%)	1 (5%)	3 (18.8%)	1 (12.5%)	0	0	0	0
Klebsiella spp.	4 (7.1%)	2 (6.5%)	2 (10%)	1 (6.3%)	1 (12.5%)	1 (16.7)	0	0	0
Citrobacter spp.	5 (8.9%)	2 (6.5%)	0	0	0	0	0	0	0
Enterobacter spp.	1 (1.8%)	0	0	3 (18.8%)	2 (25%)	2 (33.3)	0	0	0
Serratia spp.	2 (3.6%)	1 (3.2%)	1 (5%)	0	0	0	2 (40%)	0	0
Salmonella spp.	2 (3.6%)	0	0	0	0	0	0	0	0
Acinetobacter sp.	1 (1.8%)	1 (3.2%)	1 (5%)	0	0	0	0	0	0

Note. MAR, multiple antibiotic resistant; BF: Biofilm.

Table 6. Biofilm formation of Multiple Antibiotics Resistant Isolate Cultured From Ear, Nose, and Throat Infections Samples

Gram-Positive Bacteria Isolate	Ear Infection			Nose Infection			Throat Infection		
	No. of isolates (n=57)	No. of MAR (n=20)	No. of+ve BF (n=14)	No. of isolates (n=40)	No. of MAR (n=12)	No. of+ve BF (n=10)	No. of Isolates (n=18)	No. of MAR (n=11)	No. of+ve BF (n=9)
Staphylococcus aureus	19 (33.3%)	9 (45%)	9 (64.3%)	15 (37.5%)	8 (66.7%)	7 (70%)	7 (38.9%)	6 (54.5%)	4 (44.4%)
Staphylococcus spp.	11 (19.3%)	4 (20%)	4 (28.6%)	8 (20%)	3 (25%)	2 (20%)	2 (11.1%)	1 (9.1%)	1 (11.1%)
Micrococcus spp.	2 (3.5%)	2 (10%)	0	1 (2.5%)	0	0	0	0	0
Enterococcus spp.	1 (1.6%)	0	0	2 (5%)	1 (8.3%)	1 (10%)	0	0	0
Streptococcus spp.	4 (7%)	1 (5%)	0	3 (7.5%)	0	0	3 (16.7%)	2 (18.2%)	2 (22.2%)
Corynebacterium spp.	13 (22.8%)	2 (10%)	0	4 (10%)	0	0	3 (16.7%)	1 (9.1%)	1 (11.1%)
Bacillus spp.	7 (12.3%)	2 (10%)	1 (7.1%)	7 (17.5%)	0	0	3 (16.7%)	1 (9.1%)	1 (11.1%)

Note. BF: Biofilm; MAR: Multiple antibiotic resistance.

(31), implying that the potentiality of a bacteria isolate to produce biofilm contributes significantly to their degree of antibiotic resistance. However, other researchers (5,28) agreed that there is a cordial relationship between AMR and the ability to form biofilm. They also perceived that the underlying mechanisms for this relationship can be influenced by the strain of bacteria, biofilm development level, concentration of extracellular polymeric substances in biofilm, their genetic regulatory system, and the type of antimicrobial agent.

Studies have reported that cases of chronic ENT infections, especially rhinosinusitis and otitis media, are mostly caused by the formation of bacterial biofilms on adenoids (9-12). These findings are in agreement with those of our study because almost all tissue isolates are MAR producing biofilms, including 100% for Gram-positive and 83.3% for Gram-negative organisms when compared with isolates from discharge samples. This information has not only exposed one of the major causes of MAR in ENT infection, but it has further explained the reasons for the magnitude of sequels and complications of ENT infections among our patients. While other factors, such as wrong use of antibiotics, improper infection prevention and control methods, and availability of antibiotics as over-the-counter medications, are also of public health importance, more intense focus should be given to combating BPOs (2). In many parts of the world, there has been progressively increasing in-depth insight into what is being understood about the mechanism of formation, structural integrity, effects of genetics, and clinical impacts of biofilms. This also includes the ability to understand the properties of biofilm-producing bacteria to overpower potent antimicrobial agents and the methods that can be employed to impair the potency of biofilm, such as probiotic-based intervention strategies (5,27,29). Therefore, solution-oriented local studies are needed to look more into available medicinal substances and potent anti-biofilm agents that can militate against biofilms in an attempt to disarm the effect of MAR in the management of ENT infections.

Conclusion

The ability of common bacteria causing ENT infections to resist antibiotic agents is enormous and calls for urgent attention. BPOs are mostly common among the causative agents for ENT infections. These biofilm-producing bacteria have higher tendencies to dominate in body-infected tissues other than the discharges being produced; accordingly, tissue biopsy for culture and sensitivity should be considered more appropriate where visible, particularly when confronted with hard-to-treat infections in ENT clinical settings. The proper sensitivity pattern of the isolate must be effectively investigated before prescribing antibiotics to reduce the prevalence of ENT infection.

There is also an urgent need to consider anti-biofilm antibiotic agents with the development of standardized anti-biofilm protocols in the prevention and management of multiple antibiotic resistance.

Authors' Contribution

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Data curation: Oluwabusayomi Roseline Ademakinwa, Adekunle Adeyemo, Akumbu Sylvia Nwamuo.
Formal analysis: Oluwabusayomi Roseline Ademakinwa, Adekunle Adeyemo, Samuel Oluyomi Ayodele.
Investigation: Oluwabusayomi Roseline Ademakinwa, Adekunle Adeyemo, Samuel Oluyomi Ayodele, Akumbu Sylvia Nwamuo.
Methodology: Oluwabusayomi Roseline Ademakinwa, Adekunle Adeyemo, Anthonia Olufunke Oluduro.
Project administration: Oluwabusayomi Roseline Ademakinwa, Adekunle Adeyemo, Samuel Oluyomi Ayodele, Akumbu Sylvia Nwamuo, Anthonia Olufunke Oluduro.
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Visualization: Oluwabusayomi Roseline Ademakinwa, Adekunle Adeyemo, Samuel Oluyomi Ayodele, Akumbu Sylvia Nwamuo, Anthonia Olufunke Oluduro.
Writing–original draft: Oluwabusayomi Roseline Ademakinwa, Adekunle Adeyemo.
Writing–review & editing: Oluwabusayomi Roseline Ademakinwa, Adekunle Adeyemo, Samuel Oluyomi Ayodele, Anthonia Olufunke Oluduro.

Competing Interests

The authors declare that they have no conflict of interests.

Ethical Approval

This study was performed in line with the principles of the Declaration of Helsinki. Ethical approval was also granted by the Ethical and Research Committee of the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria (with protocol number: ERC/2018/06/14) before commencement of the study.

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