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Detection of Biofilm forming bacteria causing Otitis media infection

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Abstract

The current work aimed to isolate biofilm-forming bacteria causing otitis media infection and correlate with persistence and antibiotic resistance. In the beginning, sixty samples were gathered from patients who visited the ENT unit at Kirkuk General Hospital under the guidance of a medical professional. Samples were collected using cotton swabs from patients with chronic otitis media. Bacterial diagnosis were performed using manual identification methods depending on Cultural characteristic's and biochemical tests. Antibiotic susceptibility tests was conducted using ten antibiotic discs following KirbyBauer method Biofilm production test was performed quantitatively using tissue microtiter plate method. The current results showed that several types of bacteria appeared, including *Pseudomonas aeruginosa*. *Staphylococcus aureus*. *Staphylococcus epidermidis*. *Escherishia. coli* and *Proteus vulgaris*. The antibiotics assay results showed that most isolates were resistant against Ceftazidim and Cefotaxime however, Imipenem gave good inhibitions against all recording 70% susceptibility. The biofilm production results included three types: Strong biofilm producer, Medium biofilm producer. Finally: Weak biofilm producer. The biofilm strength according to isolated bacteria included Pseudomonas aeruginosa. and S. aureus recording strong production while the weak production were recorded in *E. coli* and *P. vulgaris*. In conclusion, biofilm production is associated with drug resistant and recurrent or chronic ear infection.

Introduction:

Chronic suppurative otitis media (CSOM) stands as one of the most prevalent ear infections in the human population, affecting approximately 65-330 million individuals each year. It particularly impacts children, especially those attending school, more than adults. The condition involves persistent inflammation within the middle ear and mastoid cavity, characterized by a perforated tympanic membrane accompanied by the discharge of pus. (1.2) This inflammation commonly arises following an acute otitis media episode or in the aftermath of acute upper respiratory infections. The disease's heightened occurrence is associated with a range of microorganisms, predominantly Pseudomonas spp, Staphylococcus spp, Proteus spp, and E. coli. Various external factors contribute to the spread of infection within the population, encompassing inadequate personal hygiene, overcrowding,

malnutrition, and passive exposure to smoking (3.4). Additionally, bacteria-produced factors bolster pathogenicity, promoting chronic infection by aiding bacterial invasion, disease causation, and evasion of host defenses (5.6). The formation of biofilm has been correlated with the infection's progression and resistance against antibiotic treatments (7). Surgical intervention can mitigate complications to some extent, but postoperative ear discharge remains plausible for patients.

Should the initial treatment approach yield no response, or if the patient develops conditions like cholesteatoma or other masses, referral to an otolaryngology specialist becomes crucial. In cases involving cholesteatoma, the otolaryngology team's involvement becomes essential for procedures such as mastoidectomy coupled with tympanoplasty. Furthermore, continuous assessment of hearing function and appropriate follow-up care is of paramount importance for all individuals presenting with chronic otitis media.

also the production of bacteria to biofilm help it to antibiotic resistance and have a role in influencing the cells through the direct toxic effect on the host or acting indirectly by enhancing host colonization and microbial survival , overcome the immune system(8). The wrong used of antibiotics lead to bacteria for antibiotics. posed a threat to control infection, which may lead to death or because of long time to treatment, how difficult it is to find new antibiotics that have different targets and mechanisms of action require a high cost and a decade to launch as a commercial drug prompting him to devise new ways to treat bacterial infections (9). The study aimed to isolate and detect biofilm-forming bacteria causing otitis media infection and correlate with chronic status and antibiotic resistance

Materials and Methods:

Bacterial Isolation and Identification

Sample collection

This research encompassed a group of 90 participants, with 30 of them constituting healthy controls and the remaining 60 being individuals diagnosed with chronic otitis media. The collection of samples took place at Kirkuk General Hospital in Kirkuk, Iraq, spanning from October 2022 to February 2023. Aseptic cotton swabs designated for transportation were employed to gather specimens from the site of discharge (either the right ear, left ear, or both), all under the guidance and supervision of a specialized physician. This procedure was accompanied by the completion of a questionnaire, capturing essential details such as the patient's name, age, place of residence, and medical history.

Laboratory Diagnosis

Swabs were cultured on various media types, including MacConkey agar, Blood agar, and nutrient agar. The cultures were then incubated aerobically at a temperature of 37°C for a duration of 24 hours. Subsequently, biochemical tests were performed for bacteria identification, following a specified protocol (10). For each primary positive culture, a single colony was selected, and its identification was based on various morphological attributes such as colony size, shape, color, pigment nature, translucency, lactose fermentation, edge characteristics, elevation, and texture.

To assess the cellular morphological properties of bacterial cells, Gram-stained bacterial smears were utilized. This staining method allowed examination of characteristics like Gram reaction, cell shape, arrangement, presence of spores, and more. Based on the Gram reaction results, a series of biochemical tests were carried out, including Gram staining itself, Indole test, Hydrogen peroxide test, Methyl-red test, Voges-Proskauer test, Citrate utilization test, Urease test, Motility test, Catalase Test, Oxidase Test, Kligler iron test, and Coagulase test.

Antibiotic susceptibility test

The antibiotic test was performed following Kirby/bauer diffusion method using ten antibiotics. The inhibition diameter results were recorded according to CLSI 2022 were: Levofloxacin (LEV 5 µg /disc); *S* ≥ 22, I 15−21, R≤ 14. Imipenem(IPM10 µg /disc)S ≥ 19,I 16−18,R≤15. Vancomycin(VA30 µg /disc) S ≥ 17,I 15−16,R≤14. Azithromycine(AZM15 µg /disc) S ≥ 22,I 16−21,R≤15. Gentamincin(CN10 µg /disc) S ≥ 15,I 13−14,R≤12. Cefotaxime(CTX30 µg /disc) S ≥ 26,I 23−25,R≤22. Ciproflaxacine(CIP5 µg /disc) S ≥ 25,I 19−24,R≤18. Tetracycline(TE30 µg /disc) S ≥ 19,I 15−18,R≤14. Ceftazidime(CAZ30 µg /disc) S ≥ 18,I 15−17,R≤14. Nitrofuration(F300 µg /disc) S ≥ 17,I 13−17,R≤12.

Biofilm method

The technique used to investigate biofilm formation, known as the microtiter plate assay (also referred to as a 96-well plate assay), enables the observation of bacterial attachment to a non-living surface. In this method, bacteria are cultivated within microtiter plates containing either vinyl "U"-shaped wells or other 96-well designs. After the incubation period, non-adherent planktonic bacteria are washed away, leaving only the bacteria firmly attached to the surface (forming biofilms). These adherent biofilms are then stained using crystal violet dye, allowing for clear visualization. For quantitative assessment, the stained biofilms can be dissolved and transferred to a 96-well flat-bottom plate with optical clarity, facilitating measurement using a spectrophotometer.

To prepare the initial bacterial culture, 1mL of sterile saline was introduced into a transparent polystyrene test tube with dimensions of 12×75 mm. A consistent suspension of organisms was created by transferring multiple isolated colonies from culture plates into the saline-filled tube, using sterilized cotton swabs. The density of the suspension was adjusted to match the McFarland standard specified for the test, utilizing the DensiCHEK Plus Meter (11).

Results and discussion

Sample Collection

The present research encompassed the collection of seventy specimens using cotton swabs, gathered from patients of varying ages and genders diagnosed with chronic suppurative otitis media. These samples were obtained by ENT specialists at Kirkuk Hospital. Out of these, 60 samples (86%) exhibited bacterial growth upon culturing, while 10 samples (14%) displayed no bacterial growth, this observation is consistent with previous studies (12 ,13) where findings indicated comparable percentages of 13.2% and 7.15% showing no growth. This absence of growth might be attributed to multiple factors, including the possibility of viral or fungal infections.

Identification of Bacterial Isolates

Bacterial identification involved assessing colony morphology, microscopic examination, and conducting biochemical tests. The findings were as follows: Following growth on various media, preliminary diagnoses were made based on morphological and macroscopic characteristics. The outcomes of the identified bacterial species were as follows:

From the 60 samples taken from patients with chronic otitis media, a total of 5 distinct bacterial strains were recognized. Among these, 3 strains were gram-negative bacteria: Pseudomonas aeruginosa, Escherichia coli, and Proteus vulgaris. Additionally, two types of gram-positive bacteria were detected: Staphylococcus aureus and Staphylococcus epidermidis. These bacteria are commonly associated with chronic otitis media, a condition involving inflammation or infection of the middle ear. Proper diagnosis and tailored treatment strategies are essential for the effective management of this ailment. The percentage breakdown of the diagnosed bacterial isolates is as follows:

Table 1: the percentage of isolated bacteria

Bacteria	Number	Percentage
S. aureus	20	33.3%
S. epidermidis	11	18.3%
P. aeruginosa	22	36.6%
E. coli	4	6.6%
P. vulgaris	3	%5
Total	60	100%

The study revealed the prevalence of different bacterial species causing infections in patients with chronic otitis media. The most commonly isolated species were Pseudomonas aeruginosa, accounting for 36.6% of the cases, followed by Staphylococcus aureus at 33.3%,and Staphylococcus aureus Staphylococcus epidermidis at 18.3% and Escherichia coli accounted for 6.6% respectively. Less frequently isolated species included Proteus vulgaris accounted for 5% These findings were consistent with similar studies conducted(14−16)

The results of biochemical tests for bacteria with a gram-negative classification

The outcomes of biochemical tests conducted on gram-negative bacteria are presented in the **table 2**, along with the diagnostic results from the Kligler iron agar test.

Bacteria	IMVIC Citrate test iodole, methyl red ,Voges-Proskauer test,				Catalase	Oxidase	Kligler iron agar				Motility
	IND	MR	VP	C			slant	butt	H2S	gas	
P.aeruginosa	-	-	-	+	+	+	R	R	-	-	+
E. coli	+	+	-	-	+		Y	Y	-	+	+
P. vulgaris	+	+	-	-	+	-	R	Y	+	-	+

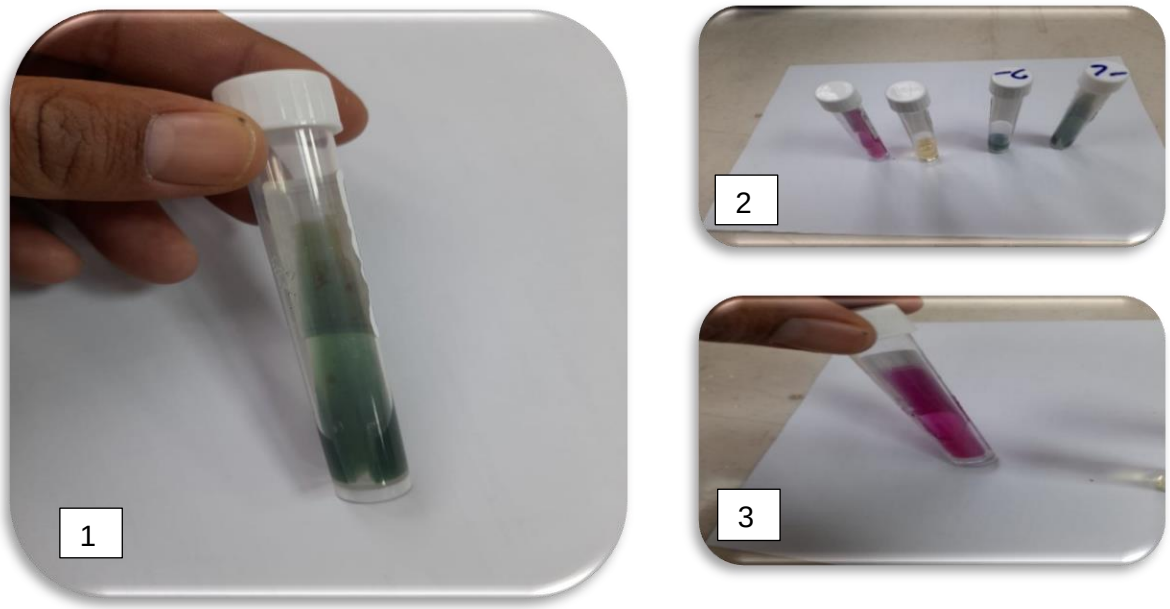


Fig. 1 Image Citrate test, Positive result: blue color, Netgative result:green color. (2) B Indole Test c: Methyl Red Test d:Voges Proskauer (3) Image uears, Positive pink red, Negative yeallow.

The outcomes of biochemical tests conducted on gram-positive bacteria

The results of diagnostic assessments for gram-positive bacteria using the oxidase, catalase, coagulase, and novobiocin tests are presented in **Table 3** Biochemical Tests Employed for the Diagnosis of Gram-Positive Bacteria.

Bacteria	Catalase	Oxidase	Coagulase	novobiocin	Hemolysin
S. aureus	+	-	+	S	+
S. epidermidis	+	-	-	S	-

S: sensitive

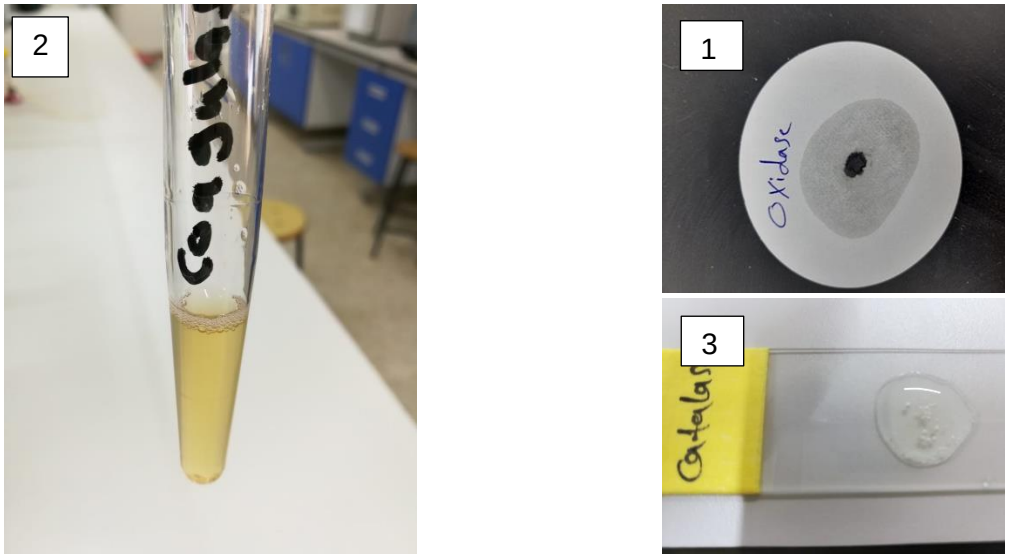


Fig. 2 Image Oxidase test, negative blak Purple color: positive, (2) coagulase positive plasma clot (3) catalase positive bubbles appear negative no bubbles appear

Result of Recovered Bacterial Species

Out of the 60 isolated samples from patients with chronic otitis media, 5 different strains of bacteria were identified. These strains include 3 types of gram-negative bacteria: Pseudomonas aeruginosa, Escherichia coli (E. coli), Proteus vulgaris, Additionally, two species of gram-positive bacteria were found: Staphylococcus aureus and Staphylococcus epidermidis. These bacteria are commonly associated with chronic otitis media, an infection or inflammation of the middle ear. Proper diagnosis and individualized treatment plans are necessary to effectively manage this condition. The study revealed the prevalence of different bacterial species causing infections in patients with chronic otitis media. The most commonly isolated species were Pseudomonas aeruginosa, accounting for 36.6% of the cases, followed by Staphylococcus aureus at 33.3%, and Staphylococcus aureus Staphylococcus epidermidis at 18.3% and Escherichia coli accounted for 6.6% respectively. Less frequently isolated species included Proteus vulgaris accounted for 5% These findings were consistent with similar studies conducted (14–16), which also reported similar bacterial species in their respective studies. likely provides a more detailed breakdown of the distribution and prevalence of these bacterial species within the study population. The study findings were in line with previous research conducted (17,18), which also identified S Pseudomonas aeruginosa, Staphylococcus aureus and Staphylococcus epidermidis as he most common bacterial species causing infections. These species showed the highest infection rates. The presence of Escherichia coli as the fourth most isolated species, accounting for 6.6% of the cases, aligned with the studies conducted (19.10). However, this differed from the results reported by(21) where E. coli ranked lowest among the isolated bacteria. The results for Proteus, Citrobacter, and Enterobacter were consistent with (17). who found 4% for Citrobacter spp and 5% for Proteus spp in 187 samples. They also reported a lower occurrence of Enterobacter spp at 3%. Conversely, these findings did not align with (22).

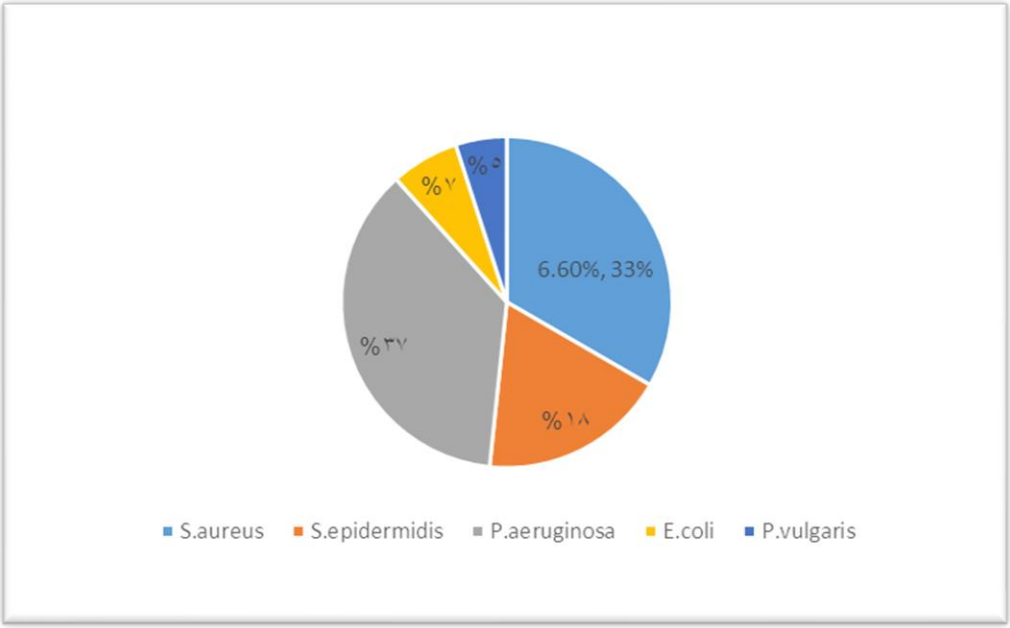


Fig. 3 The percentage of isolated bacteria

Results of Age Relationship with Chronic Suppurative Otitis Infection The age of

The data presented reveals varying infection rates among different age groups. The highest percentage of infections, at 35%, was observed in the age group of above 21 -40 years. Age groups 41-60 years followed closely behind with an infection rate of 30%, while age groups 0-20 had a rate of 20%. Infection rates gradually decreased with increasing age, with age groups 61-80 showing a rate of with previous studies conducted cases 58% were males and 42% females. By, Conclusion The percentage at 35%, was observed in the age group of above 21 -40 years.of men in the result of exposure to loud sound explosion or swimming in unclean places.

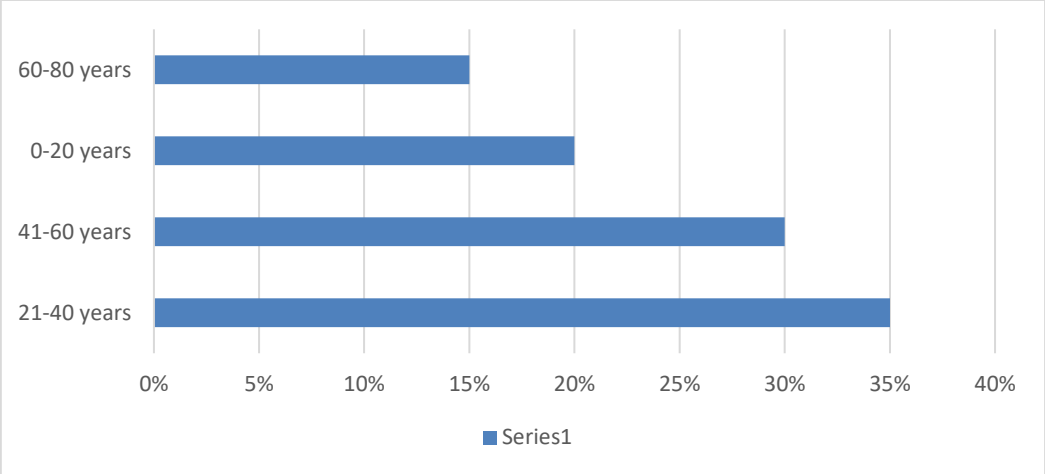


Fig. 4 The percentage of age

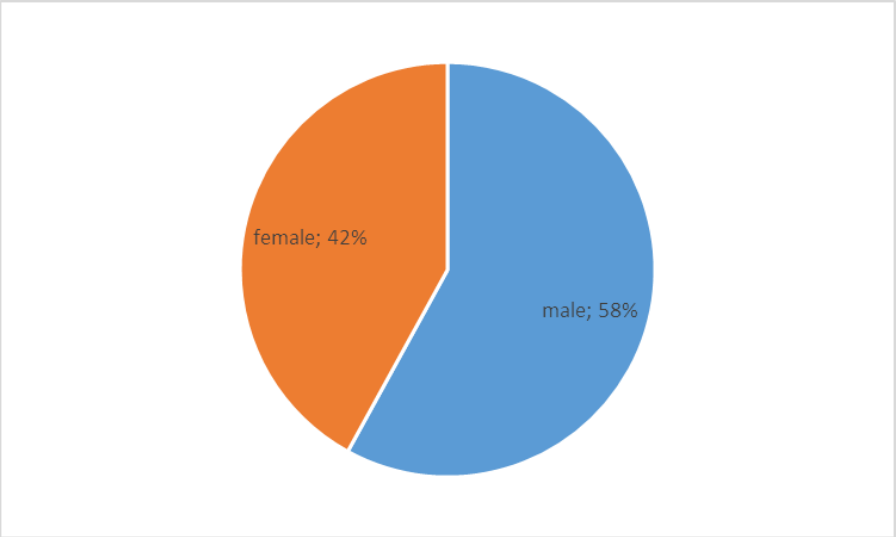


Fig. 5 percentage of man and waman.

Consistency in results was observed across various age groups (23–25). The study group comprised patients who exhibited complaints of unilateral or bilateral ear discharge, those who granted consent for participation, individuals below the age of 100 years, and those above the age of 1 year. Patients aged over 100 years or under 1 year, lacking ear discharge, and those with outer ear anomalies such as complete stenosis or atresia of the external auditory canal, were not included. Enrolled patients were informed about the entire study procedure in a language they understood.

A comprehensive history was obtained from study participants, covering aspects such as the nature of ear discharge, whether it was unilateral or bilateral, discharge duration, presence of earache, diminished hearing, tinnitus, history of hearing loss, and prior surgical interventions. A thorough clinical examination was conducted, encompassing a general physical assessment and systemic examination to evaluate the overall health of participants. This examination extended to an ear, nose, and throat (ENT) evaluation, involving thorough inspections of the ears, throat, and nose.

Samples of aural discharge were collected using sterile culture sensitivity tubes and then sent to the microbiological laboratory at Regional Hospital Bilaspur (SRL Diagnostics). Culture sensitivity reports were subsequently collected from patients during their follow-up visits.

To summarize, the data indicates that younger age groups, especially children, are more susceptible to infections due to factors such as suboptimal hygiene practices, exposure to contaminated objects or soil, transmission among students, less mature immune systems, and the anatomical characteristics of their eustachian tubes. A significant proportion of these individuals reside in rural areas (53.3%), while the remaining portion (46.7%) resides in urban areas.

Antibiotic Susceptibility Test:

Sensitivity test for bacteria isolated from CSOM as shown in **table 4** The resistance of isolates to antibiotics is under study

Antimicrobial agent and disc concertation	S. aureus N=20 N(%)	P. aeruginosa N=22 N(%)	S. epidermidis N=11	P. vulgari N=3	E. coli n=4	Total
Levofloxacim(5)	7(36)	11(50)	6(54)	3(100)	2(50)	48
Imipenem(10)	7(36)	18(80)	10(86)	3(100)	4(100)	70
Ciprofloxacin(5)	4(18)	8(35)	7(63)	3(100)	2(50)	40
Azithromycin(15)	2(9)	7(30)	3(27)	0(00)	1(25)	21
Gentamicin(10)	4(18)	10(45)	6(54)	1(33)	1(25)	36
Vancomycin(30)	4(18)	6(25)	4(36)	0(00)	0(00)	23
Cefotaxime(30)	4(18)	0(00)	1(09)	0(00)	0(00)	8
Ceftazidim(30)	7(36)	2(10)	1(09)	0(00)	0(00)	16
Nitrofuration(300)	5(27)	6(25)	4(36)	0(00)	0(00)	25
Tetracycline(30)	4(18)	4(20)	4(36)	0(00)	1(25)	21

In the ongoing study, the findings regarding bacterial resistance and sensitivity are as follows:

For **S. aureus**:

- Resistance: Levofloxacin (63%), Imipenem (63%), Vancomycin (81%), Gentamicin (81%), Azithromycin (90%), Cefotaxime (81%), Ciprofloxacin (81%), Tetracycline (63%), Nitrofurantoin (72%), Ceftazidime (90%).

- Sensitivity: Levofloxacin (54%), Imipenem (86%), Gentamicin (54%), Ciprofloxacin (63%).

- Resistance: Vancomycin (63%), Azithromycin (73%), Cefotaxime (86%), Nitrofurantoin (68%), Ceftazidime (86%), Tetracycline (68%).

For **P. aeruginosa**:

- Sensitivity: Levofloxacin (50%), Imipenem (80%).

- Resistance: Vancomycin (75%), Azithromycin (70%), Cefotaxime (100%), Ciprofloxacin (65%), Ceftazidime (100%), Tetracycline (80%), Gentamicin (55%), Nitrofurantoin (75%).

For **P. vulgaris**:

- Sensitivity: Levofloxacin (100%), Imipenem (100%), Ciprofloxacin (100%).

- Resistance: Vancomycin (100%), Azithromycin (100%), Cefotaxime (100%), Ceftazidime (100%), Tetracycline (100%), Gentamicin (65%), Nitrofurantoin (100%).

For **E. coli**:

- Sensitivity: Levofloxacin (50%), Imipenem (100%), Ciprofloxacin (50%).

- Resistance: Vancomycin (100%), Azithromycin (75%), Cefotaxime (100%), Ceftazidime (100%), Tetracycline (75%), Gentamicin (75%), Nitrofurantoin (100%).

The study outcomes reveal that S. aureus exhibited resistance to imipenem, gentamicin, ceftazidime, and ciprofloxacin. These results are consistent with previous findings (26) where S. aureus strains obtained from COME samples displayed reduced susceptibility to antibiotics compared to other pathogens. This aligns with references (27, 28) as well. The study also identified that P. aeruginosa demonstrated sensitivity to levofloxacin and imipenem, corroborating with previous research (29) and most other studies. Despite being of the same drug class, gentamicin was not as effective as amikacin in numerous studies, and piperacillin-tazobactam, ceftazidime, and ciprofloxacin exhibited diminished sensitivity in multiple studies (30).

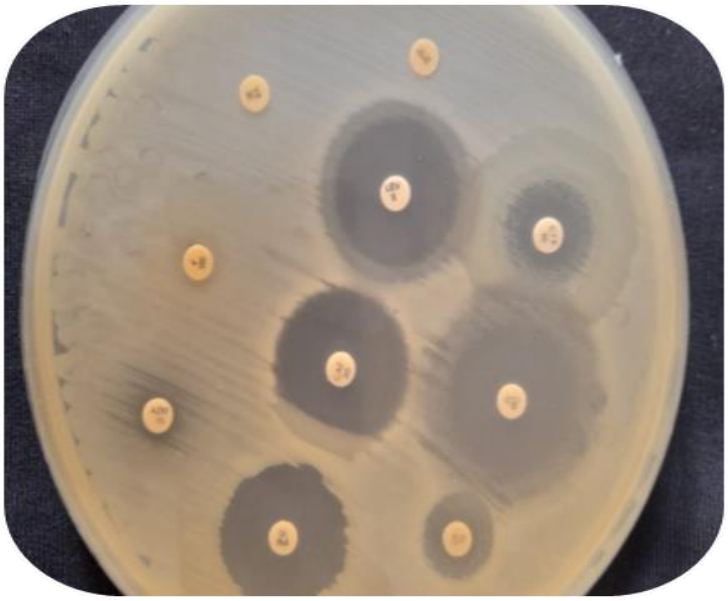


Fig. 6 sensitive antibiotic

Quantitative Biofilm Formation Assay

The capacity for biofilm formation among 60 distinct bacterial isolates was assessed using the 96-well microtiter plate assay, employing the crystal violet staining technique. The findings indicated that both gram-positive and gram-negative bacteria exhibited the ability to produce biofilms. Notably, S. aureus, P. aeruginosa, S. epidermidis, and Proteus spp. were identified as having the capability to generate biofilms. The obtained results were categorized into four groups based on their respective optical densities, as follows:

(3) Strong biofilm producer ($4 \times ODc < OD$)

(2) Medium biofilm producer ($2 \times ODc < OD \leq 4 \times ODc$)

(1) Weak biofilm producer ($ODc < OD \leq 2 \times ODc$)

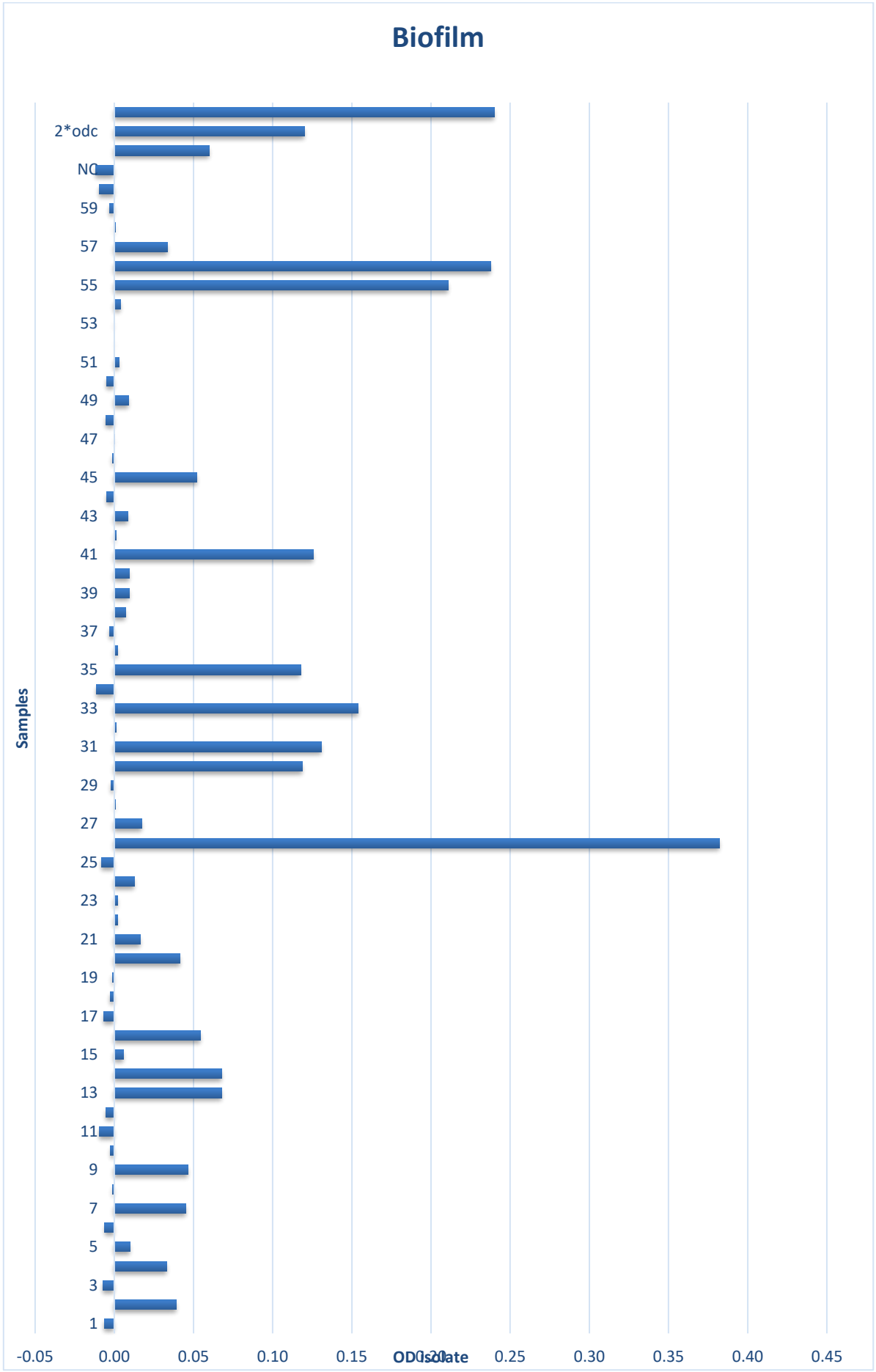
(0) non-biofilm producer ($OD \leq ODc$

S. aureus and P. aeruginosa findings were consistent with reference (31), while Proteus spp. results aligned with reference (32). The presence of biofilm-producing capability was confirmed for isolated S. epidermidis, which corroborates reference (33) indicating S. epidermidis isolates' biofilm production. Additionally, agreement was found with reference (34), which indicated the biofilm-forming capacity of Proteus spp.

In contrast, the study's results diverged from references regarding Pseudomonas spp. and E. coli isolates. In this study, it was observed that 50% of E. coli and 90% of Pseudomonas spp. were non-biofilm producers, aligning with the current findings. Furthermore, the present study contrasted with prior research, particularly concerning P. aeruginosa and S. epidermidis isolates, where the previous study noted a 100% absence of biofilm formation. Among the S. aureus isolates, a significant portion were found to be non-biofilm producers.

Samples	OD1	OD2	OD3	Average	OD isolate	odc	2*odc	4*odc	Results
1	0.06	0.05	0.05	0.05	-0.01	0.06	0.12	0.24	0
2	0.12	0.09	0.09	0.10	0.04	0.06	0.12	0.24	0
3	0.05	0.05	0.05	0.05	-0.01	0.06	0.12	0.24	0
4	0.08	0.10	0.10	0.09	0.03	0.06	0.12	0.24	0
5	0.06	0.06	0.08	0.07	0.01	0.06	0.12	0.24	0
6	0.05	0.06	0.05	0.05	-0.01	0.06	0.12	0.24	0
7	0.13	0.10	0.09	0.11	0.05	0.06	0.12	0.24	0
8	0.06	0.06	0.07	0.06	0.00	0.06	0.12	0.24	0
9	0.07	0.07	0.19	0.11	0.05	0.06	0.12	0.24	0
10	0.05	0.06	0.06	0.06	0.00	0.06	0.12	0.24	0
11	0.05	0.05	0.05	0.05	-0.01	0.06	0.12	0.24	0
12	0.05	0.06	0.06	0.05	-0.01	0.06	0.12	0.24	0
13	0.12	0.13	0.14	0.13	0.07	0.06	0.12	0.24	1
14	0.10	0.13	0.15	0.13	0.07	0.06	0.12	0.24	1
15	0.07	0.07	0.06	0.07	0.01	0.06	0.12	0.24	0
16	0.09	0.13	0.12	0.11	0.05	0.06	0.12	0.24	0
17	0.05	0.05	0.05	0.05	-0.01	0.06	0.12	0.24	0
18	0.06	0.05	0.06	0.06	0.00	0.06	0.12	0.24	0
19	0.06	0.06	0.06	0.06	0.00	0.06	0.12	0.24	0
20	0.09	0.11	0.10	0.10	0.04	0.06	0.12	0.24	0
21	0.10	0.06	0.07	0.08	0.02	0.06	0.12	0.24	0
22	0.07	0.06	0.06	0.06	0.00	0.06	0.12	0.24	0
23	0.07	0.06	0.06	0.06	0.00	0.06	0.12	0.24	0
24	0.07	0.08	0.07	0.07	0.01	0.06	0.12	0.24	0
25	0.05	0.05	0.05	0.05	-0.01	0.06	0.12	0.24	0
26	0.39	0.42	0.52	0.44	0.38	0.06	0.12	0.24	3
27	0.09	0.08	0.07	0.08	0.02	0.06	0.12	0.24	0
28	0.07	0.06	0.06	0.06	0.00	0.06	0.12	0.24	0
29	0.06	0.06	0.06	0.06	0.00	0.06	0.12	0.24	0
30	0.09	0.27	0.18	0.18	0.12	0.06	0.12	0.24	2
31	0.26	0.21	0.10	0.19	0.13	0.06	0.12	0.24	2
32	0.06	0.06	0.06	0.06	0.00	0.06	0.12	0.24	0
33	0.20	0.23	0.21	0.21	0.15	0.06	0.12	0.24	2
34	0.05	0.05	0.05	0.05	-0.01	0.06	0.12	0.24	0
35	0.18	0.18	0.17	0.18	0.12	0.06	0.12	0.24	2
36	0.06	0.07	0.06	0.06	0.00	0.06	0.12	0.24	0
37	0.06	0.06	0.06	0.06	0.00	0.06	0.12	0.24	0
38	0.08	0.06	0.06	0.07	0.01	0.06	0.12	0.24	0
39	0.05	0.08	0.07	0.07	0.01	0.06	0.12	0.24	0
40	0.07	0.07	0.07	0.07	0.01	0.06	0.12	0.24	0
41	0.25	0.13	0.18	0.19	0.13	0.06	0.12	0.24	2
42	0.06	0.06	0.06	0.06	0.00	0.06	0.12	0.24	0
43	0.07	0.07	0.07	0.07	0.01	0.06	0.12	0.24	0

44	0.06	0.06	0.05	0.06	0.00	0.06	0.12	0.24	0
45	0.14	0.13	0.07	0.11	0.05	0.06	0.12	0.24	0
46	0.06	0.06	0.06	0.06	0.00	0.06	0.12	0.24	0
47	0.06	0.06	0.06	0.06	0.00	0.06	0.12	0.24	0
48	0.05	0.06	0.05	0.05	-0.01	0.06	0.12	0.24	0
49	0.08	0.06	0.07	0.07	0.01	0.06	0.12	0.24	0
50	0.06	0.06	0.05	0.06	-0.01	0.06	0.12	0.24	0
51	0.06	0.07	0.06	0.06	0.00	0.06	0.12	0.24	0
52	0.06	0.06	0.06	0.06	0.00	0.06	0.12	0.24	0
53	0.06	0.06	0.06	0.06	0.00	0.06	0.12	0.24	0
54	0.06	0.07	0.06	0.06	0.00	0.06	0.12	0.24	0
55	0.16	0.14	0.52	0.27	0.21	0.06	0.12	0.24	2
56	0.20	0.25	0.44	0.30	0.24	0.06	0.12	0.24	3
57	0.07	0.07	0.15	0.09	0.03	0.06	0.12	0.24	0
58	0.06	0.06	0.06	0.06	0.00	0.06	0.12	0.24	0
59	0.06	0.06	0.05	0.06	0.00	0.06	0.12	0.24	0
60	0.05	0.05	0.05	0.05	-0.01	0.06	0.12	0.24	0
NC	0.05	0.05	0.05	0.05	-0.01	0.06	0.12	0.24	0


Biofilm
2*odc
NC
59
57
55
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31
29
27
25
23
21
19
17
15
13
11
9
7
5
3
1
Samples
-0.05
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
OD value

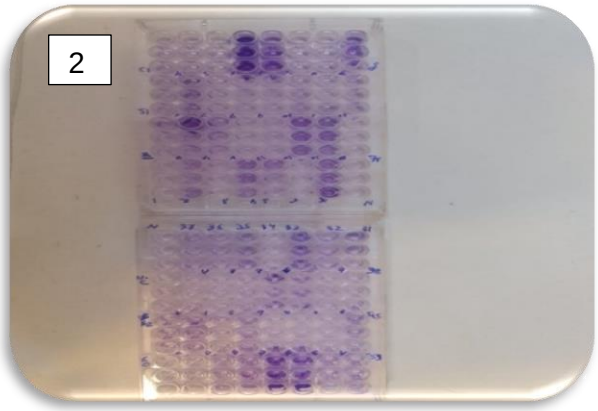

Fig. 7 after dyeing

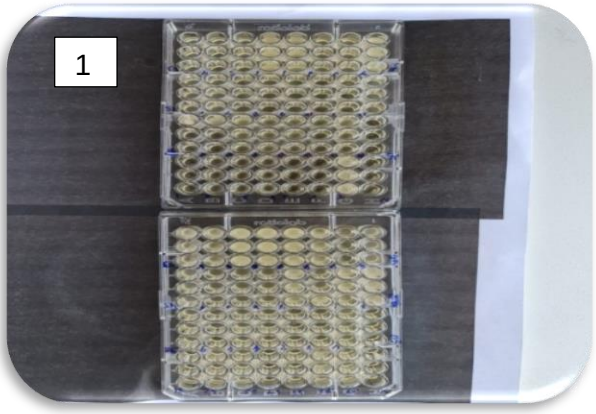

Fig. 8 before dyeing

Statistics:

Statistics were performed in excel in the usual way

Conclusion:

The research revealed the prevalence of Pseudomonas spp. and S. aureus strains compared to other isolates. The antibiotic susceptibility analysis indicated resistance to multiple treatments, although Imipenem exhibited effective inhibition against a majority of isolates. Biofilm assessment yielded strong positive outcomes for S. aureus and Pseudomonas aeruginosa.

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الكشف عن البكتيريا المكونة للغشاء الحيوي المسببة لعدوى التهاب الأذن الوسطى

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التهاب الأذن الوسطى، البكتيريا، الغشاء الحيوي، المضادات الحيوية

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الخلاصة:
يهدف العمل الحالي إلى عزل البكتيريا المكونة للغشاء الحيوي المسببة لعدوى التهاب الأذن الوسطى وربطها بمقاومة المضادات الحيوية ومقاومة المضادات الحيوية. في البداية تم جمع ستين عينة من المرضى الذين يحضرون إلى مستشفى كركوك العام في الوحدة الانف والاذن والحنجرة تحت إشراف الطبيب. تم جمع العينات باستخدام مسحات قطنية من مرضى التهاب الأذن الوسطى المزمن. تم إجراء التشخيص البكتيري باستخدام طرق التعريف اليدوية اعتمادًا على الخصائص المزرعية والاختبارات البيوكيميائية. تم إجراء اختبارات الحساسية للمضادات الحيوية باستخدام عشرة أقراص من المضادات الحيوية باتباع طريقة كيربيباور تم إجراء اختبار إنتاج بايوفلم كميًا باستخدام طريقة الصفائح الدقيقة. أظهرت النتائج الحالية ظهور عدة أنواع من البكتيريا، بما في ذلك الزائفة الزنجارية. المكورات العنقودية الذهبية. المكورات العنقودية البشروية *Escherishia. coli* و*Proteus vulgaris*. أظهرت نتائج فحص المضادات الحيوية أن معظم العزلات كانت مقاومة ضد السيفتازيديم والسيفوتاكسيم، إلا أن عقار الإيميبينيم أعطى مثبطات جيدة ضد جميع العزلات التي سجلت حساسية 70٪. اشتملت نتائج إنتاج الأغشية الحيوية على ثلاثة أنواع: منتج بايوفلم قوي، ومنتج بيوفيلم متوسط. أخيرًا: منتج ضعيف للبايوفلم. تضمنت مقاومة الأغشية الحيوية وفقًا للبكتيريا المعزولة الزائفة الزنجارية. والمكورات العنقودية الذهبية سجلت إنتاجًا قويًا بينما تم إعادة ترميز الإنتاج الضعيف في *E.coli* و *P. vulgaris*. في الختام، يرتبط إنتاج البايوفيلم بمقاومة الأدوية وعدوى الأذن المزمنة أو المتكررة.