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Prevalence and Antibiotic Sensitivity Profile of *Pseudomonas aeruginosa* Isolated from Patients with Otitis Media in Kirkuk City

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A B S T R A C T

Background: Otitis media [OM] is a middle ear inflammation. It is a prevalent medical disorder that affects people of all ages and both genders. Pseudomonas aeruginosa [P. aeruginosa] is the most prevalent pathogen bacteria responsible for the disease and has a high level of antibiotic resistance. The middle ear mucosa is destroyed by P. aeruginosa because of its virulence characteristics. Antibiotic resistance constitutes the main threat to people's health.

Objective: The objective of the current study is to evaluate the frequency of P. aeruginosa isolated from Otitis media patients and investigate their antibiotic susceptibility pattern. Materials and Methods: All 235 ear swabs were collected from otitis media patients who visited a specialized outpatient center in Kirkuk City and the ENT consultation facility at Kirkuk Hospitals. Both the antibiotic susceptibility which was tested by the VITEK 2 AST card and bacterial isolation and identification were done.

Results: The results of this study reveal that out of 235 ear swabs collected from patients with otitis media showed 22/235 [9.4 %] isolation rate for P. aeruginosa. And among the most effective antibiotics against of the detected pathogenic bacteria were Amikacin [95.4%], followed by Ceftazidime/ Avibactam, Piperacillin /Tazobactam and Ceftolozane/ Tazobactam each with [91%], Colistin [86%], Imipenemand and Meropenem [81.8%, 86.4%] respectively, Ceftazidime, Cefeime, Levofloxacin and Azreonam each with [77.3%], Ciprofloxacin and Ofloxacin both [72.7%] with Gentamicin [68.2%].

Introduction

Otitis media [OM] is often known as middle ear infections, the term "middle ear infection" refers to a collection of complicated inflammatory disorders caused by pathogens that damage the middle ear cavity, either they are bacterial or viral [1]. It is one of the most prevalent disorders of the ears among elderly people. The eustachian tube, which links the middle ear to the throat, frequently becomes malfunctioning and results in OM, eustachian tube obstruction inhibits normal drainage, resulting in an accumulation of middle ear fluid behind the eardrum, the building up fluid serves as a culture medium for the growth and development of the bacteria and viruses that cause acute OM [2-4]. The most frequent bacterial pathogens that cause OM is *Pseudomonas aeruginosa* [*P. aeruginosa*] [5]. *P. aeruginosa* is a member of the common Pseudomonadaceae family of aerobic, non-fermenting gram-negative organism that can survive in a variety of environments [6]. In addition, this organism is known to be the most common gram-negative species of bacteria associated with significant rates of hospital-acquired infections in various locations [7]. It is becoming more and more resistant to several drugs, these isolates that are multidrug-resistant are made available by this resistance [8]. MDR *P. aeruginosa* strain is resistant to three or more types of antibiotics from the groups of Penicillins, Cephalosporins, Monopactam, Carbapenems, Fluoroquinolones and Aminoglycosides [9]. Furthermore, a rise in the prevalence of multidrug resistance [MDR] in this bacteria has been noted in association with increasing mortality and morbidity [10]. Antibiotic resistance, which affects treatment choices, is a problem for world health, and this bacteria may be resistant to antibiotics by a variety of ways, involving: the target area mutation, diminished antibiotic entry, antimicrobial efflux and drug inactivation by bacterial enzymes [11]. For this reason, it is essential for generating novel and effective antimicrobial therapies [12]. Previously, antibiotics have been the final barrier of defense against life-threatening infections caused by *P. aeruginosa*. To reduce the prevalence of MDR strains, it is essential to concentrate on identifying the origin of infections [13].

Materials and Methods

Specimens

The research was carried out in Kirkuk City in the period between November 2022 and May 2023. Total of 235 ear swabs were collected from Otitis Media [OM] patients who visited the Ear, Nose and Throat Department [ENT] at Azadi Teaching Hospital, Kirkuk General Hospital and outpatient clinics. Also, it is collected with the support from specialized doctors who used a sterile swab to collect these samples.

Isolation and identification

All of 235 ear swabs were cultured on Blood agar, MacConkey agar and Cetrimide agar. The colonies that developed were tested for oxidase production, catalase test, gram staining, phenotypic characteristics and biochemical reactions for accurate strain identification by employing the analytical profile index 20E [API 20E] and VITEK® 2 GN ID card. These were used for the purpose of assessing *P. aeruginosa* susceptibility and resistance, antibiotic susceptibility tests were performed through using a VITEK® 2 AST card. Large, gray to black colonies of *P. aeruginosa* can be seen on blood agar and result in a unique zone that is characteristic of blood hemolysis type B [hemolytic], which is brought on by the production of hemolysin. These isolates have been cultivated on Cetrimide agar as a selective medium for these bacteria to differentiate it from other species. In MacConkey agar, which is a non-lactose fermenter, they appear pale and they are capable of surviving in a wide range of temperatures from 4 to 42 °C [14].

Table 1. Morphological and biochemical tests for identification of P. *aeruginosa*

Test	Gram stain	Catalase	Oxidase	Citrate	Motility	VP	MR	Urease
Results	-ve	+	+	+	+	-	-	-

*[G –ve]; Gram negative; [+]: positive; [-]: negative; [MR]: methyl red; [VP]: Voges-Proskauer

Antibiotic susceptibility testing [AST]

Whenever applied in accordance with the instructions, the VITEK 2 gram-negative susceptibility card is designed for use with the VITEK 2 Systems in clinical laboratories just like an in vitro test for identifying the susceptibility of significant aerobic gram-negative bacilli to antimicrobial drugs.

The steps in the procedure below are general information that applies to all susceptibility products.

1. Sub-culturing the organism that will be tested on MacConkey agar and incubating at 35 c to 37c for 18 to 24 hours are the first steps of producing a suspension from pure culture and selecting isolated colonies.
2. Transfer 3.0 ml of sterile saline [aqueous 0.45% to 0.50% Na CL, ph 4.5 to 7.0] in an aseptic manner into a polystyrene test tube that is 12 mm by 75 mm in size.
3. Prepare a homogenous organism suspension with a density that complies with the appropriate McFarland standards [0.50 to 0.63] via sterile technique.
4. Transfer 145 ml of the suspension produced during step 3 into a second tube that contained 3.0 ml of saline for manual dilution [VITEK 2 Compact], after that, insert this tube into the cassette along with a susceptibility card, the initial bacterial suspension tube can also be used to inoculation an identification card.
5. Quality control organisms carried out according to the test card prepared.
6. The instrument records the evolution of each well on the card over a specified period of time [up to 18 hours for Gram-negatives], the minimum inhibitory concentration [MIC] values [or results of tests, as suitable], for each antimicrobial listed on the card are determined at the final stage of the incubation cycle.

Statistical Analysis

A computerized statistical analysis was performed with SPSS [Statistical Package for Science Services]. Chi-square [X2] and probability [P value] are employed to compare, and a P value of 0.05 or less was considered statistically significant [S]. A P value of 0.01 or less was considered highly significant [H.S.] and a P value more than 0.05 or was considered non-significant [N.S.].

Results and Discussions

Isolation and identification

After accumulating samples and completing all the diagnostic procedures, 110 samples of positive growth at a rate of [46.8%] after growth on specialized culture media, while 125 samples at a rate of 53.2% showed no growth. This study's results, as shown in the Table [2], present that a p-value = 0.166 which is not significant. This study is similar to those of Çetin et al., [15] within its 1290 swab culture. Bacterial growth was found in [47%] of cases while it was absent growth in 53%, as opposed to study [16]. Bacterial growth was detected in 83.4% of the 36 ear swabs, while 11.1% demonstrated no growth. The cause of negative growth may be attributed to other organisms such as a virus, fungus or anaerobic bacterial type which is able to be identified using different techniques. For this reason, the media, which is used to cultivate, does not meet the necessary requirements for its growth. Figure [1]. Figure [2].When compared to the isolation rate of other bacterial species, which constitute 80/102 [78.4%], *P. aeruginosa* was one of the dominant species, accounting for 22/102 [21.6%] of the total, and show a highly significant statistical relationship between bacteria presence and types of other bacteria with a p-value = 0.000, as show in the Table [3]. *P. aeruginosa* was the most prevalent cause of otitis medium with a high ratio of isolation, according to the findings of the current investigation. It is regarded as a common bacterium that may persist in a variety of conditions, acquires persistent antibiotic resistance mechanisms, competes with other species, and is present in swimming pools [17]. This result close to a study [16] that reported *P. aeruginosa* is predominated among the species, accounting for 11/30 [36.7%], while the other bacterial species were isolated 19/30 with [63.3%] and [18] reported that the positive culture of *P. aeruginosa* was 65/210 samples [30.95%]. The changes in bacterial type percentages are affected by geographical areas, factors affecting the environment and personal health conditions [19].

Table 2. Comparison between the samples with bacterial growth and no bacterial growth from OM, chi- square test was used and p-value at level of 0.05 was regarded significant, the level p-value was 0.166.

Sample Culture	No.	%	p-value
Bacterial Growth	110	46.8%	
No Bacterial Growth	125	53.2%	0.166
Total	235	100%	

Table 3. Proportion of P. aeruginosa and others bacterial species in ear swabs.

Bacterial Species	No.	Percentage	p- value
P. aeuginosa	22	21.6%	
Others Bacteria	80	78.4 %	0.000
Total	102	100%	

Chi- square test was used and p- value at level of 0.05 regarded significant .

Figure 1. Cultural characteristics of P. aeruginosa on (A) MaCconkey agar and (B) Blood Agar

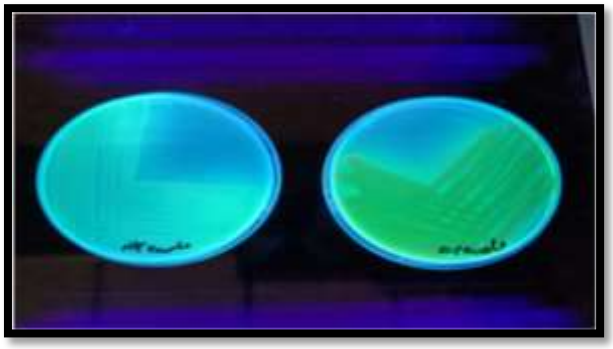

Figure 2. Culture characteristics of P. aeruginosa on cetrimide agar under UV light

Antibiotic susceptibility test for P. *aeruginosa* isolates

In the present study antibiotic susceptibility test had been done for all isolates, P. aeruginosa isolates were resistant 100% to [Amoxicillin, Ampicillin, Amoxicillin /Clavulanic Acid, Ertapenem, Cefuroxime, Cefixime and Ceftriaxone], [9.1%] and [13.6%] was resistance intermediately to Imipenem and Meropenem respectively, and highest susceptibility to Amikacin [95.4%], followed by Ceftazidime/ Avibactam, Piperacillin /Tazobactam and Ceftolozane/ Tazobactam each with [91%], Colistin [86.4%], Imipenem and Meropenem [81.8%, 86.4%] respectively, Ceftazidime, Cefepime, Levofloxacin, and Aztreonam all with [77.3%], Ciprofloxacin and Ofloxacin both [72.7%] with Gentamicin [68.2%], according to the table [4], statistical analysis on antibiotic susceptibility was very significant [P< 0.05]. According to antibiotic classes sensitivity results documented a high sensitivity of P. aeruginosa isolates to Cephalosporine [29.4%], followed by Fluoroquinolone with [19.5%] and Carbapenem, Aminoglycosides, Penicillin, Polymyxin and B-lactam with [14.7%, 14.3%, 7.9%, 7.5% and 6.7%] respectively, and P. aeruginosa showed high resistance to Cephalosporins [39%], followed by Penicillin

[33.2%], while resistant to Carbapenem [11.7%], and resistant to Fluoroquinolone, Aminoglycosides, B-lactam and Polymyxin with [8.3%, 3.9%, 2.4% and 1.5%]. respectively as in Table [5].

Table 4. Antibiotics susceptibility tests of P. *aeruginosa*.

No.	Antibiotics	R	%	I	%	S	%	T	Drugs classes
1	Amoxicillin	22	100	0	0	0	0	22	
2	Ampicillin	22	100	0	0	0	0	22	
3	Amoxicillin/ Clavulanic	22	100	0	0	0	0	22	Penicillin
4	Piperacillin/ Tazobactam	2	9	0	0	20	91	22	
5	Aztreonam	5	22.7	0	0	17	77.3	22	B-lactam
6	Ertapenem	22	100	0	0	0	0	22	
7	Imipenem	2	9.1	2	9.1	18	81.8	22	Carbapenem
8	Meropenem	0	0	3	13.6	19	86.4	22	
9	Cefuroxime	22	100	0	0	0	0	22	
10	Cefixime	22	100	0	0	0	0	22	
11	Ceftazidime	5	22.7	0	0	17	77.3	22	
12	Ceftriaxone	22	100	0	0	0	0	22	
13	Ceftazidime/ Avibactam	2	9	0	0	20	91	22	
14	Ceftolozane/ Tazobactam	2	9	0	0	20	91	22	Cephalosporine
15	Cefepime	5	22.7	0	0	17	77.3	22	
16	Amikacin	1	4.6	0	0	21	95.4	22	Aminoglycoside
17	Gentamicin	7	31.8	0	0	15	68.2	22	
18	Ciprofloxacin	6	27.3	0	0	16	72.7	22	
19	Levofloxacin	5	22.7	0	0	17	77.3	22	Fluoroquinolone
20	Ofloxacin	6	27.3	0	0	16	72.7	22	
21	Colistin	3	13.6	0	0	19	86.4	22	Polymyxin
	Total	205		5		252			

Table 5. Distribution of antibiotic resistance of *P. areuginosa* according to antibiotic classes

Antibiotics categories	Resistant %	Sensitive%	Intermediate%
Penicillin	33.2%	7.9%	0
B-lactam	2.4%	6.7%	0
Carbapenem	11.7%	14.7%	0.5%
Cephalosporine	39%	29.4%	0
Aminoglycosides	3.9%	14.3%	0
Fluoroquinolone	8.3%	19.5%	0
Polymyxin	1.5%	7.5%	0
Total	100%	100%	

R: Resistance, I: Intermediate, S: Sensitivity, %: Percentage, T: Total.

In this study *P. aeruginosa* isolates were 100% MDR, this result disagreement with [20] who reported that MDR *P. aeruginosa* was isolated constituted 10% of all isolates. Antibiotic sensitivity results for present study showed that this isolates showed high sensitivity to Meropenem and Imipenem [Carbapenems] at [86.4% and 81.8%] respectively, this study agree [18] who showed that [96.92%] were sensitive to Imipenem and Meropenem with [93.84%]. The structure of the antibiotics Penems (Meropenem and Imipenem) differs slightly from that of Penicillin, these antibiotics are more active against both gram-positive and gram-negative bacteria, consequently, they are more resistant to beta-lactamase hydrolysis [21]. The current study found that the sensitivity to Ciprofloxacin 72.7%, Levofloxacin 77.3% and Ofloxacin 72.7%, and high sensitive to Amikacin at [95.4%] and sensitive to Gentamicin at [68.2%], these results agree with [22] it showed sensitive to Gentamicin [95.35%], Amikacin [88.37%], Levofloxacin [76.74%], Ciprofloxacin [67.44%], Ofloxacin [51.16%] and disagree with [20] shows the *P. aeruginosa* isolates were resistant to Levofloxacin [42.5%], Ciprofloxacin [40%]. This study reported high sensitivity to Colistin [86.4%], Piperacillin /Tazobactam [91%], Ciprofloxacin [72.7%], and Cefepime [77.3%], the results close to [15] who report the *P. aeruginosa* strains grown in ear swabs cultures were most susceptible to Colistin [75%] and Piperacillin/Tazobactam [66%] and disagree with [23] show that these organisms were resistant to Cefepime [58%], and [24] who report sensitivity of *P. aeruginosa* to Ciprofloxacin was active against all numbers of cases expect 5 cases of students have *pseudomonas* resistance to Ciprofloxacin, followed by Amikacin, Gentamycin, Ceftazidime and Ceftriaxone, the

present study disagree with [24]. Research done in Pakistan [25] found that *P. aeruginosa* has a higher prevalence of Fluoroquinolone resistance [48.7%], followed by antipseudomonal Penicillin [41.7%], and Carbapenem resistance [29.4%], the present study not close to [25]. The present study reported that a higher prevalence of Cephalosporine resistance (39%), followed by Penicillin [33.2%], and low prevalence of Fluoroquinolone resistance [8.3%], and resistant to Carbapenem [11.7%], and this study agree with [26] demonstrate that all *P. aeruginosa* isolates tested in the study were resistant to Amoxicillin and Amoxicillin/Clavulanate and this may be due to the misuse of antibiotics, in our study result of resistance is higher than the rate of resistance reported by Ahmad et al., [25] in Pakistan where the resistance to Amoxicillin and Amoxicillin/Clavulanate was 73.4% and 67.7% respectively, and his may be supported by the time difference between the investigations, in addition to the variations in the drugs and strains used and most commonly utilized drugs, however, during this study, demonstrated that high level of resistance to many different antibiotics, which is consistent with numerous studies [27,15], the result of present study showed highest resistance to Cephalosporine generations [39%], this agree with [18] exhibited a strong resistance to Cephalosporine generations, Cephalosporin resistance is regarded as a signal to stop taking antibiotics at random, in part because of the fact that these organizations are regarded as strong anti-Pseudomonals.

Resistance can be caused by different methods, such as: [28, 29, and 30].

- Antimicrobial agents are unable to inhibit microorganisms because the majority of them are inactive during metabolism in the biofilm's layers that are deeper.
- Antimicrobial compounds are ineffective because they are removed from the biofilm by bacterial populations through "efflux action".
- Antimicrobial drug concentrations are subnormal.
- Antibiotics are unable to pass through the thick coat.
- The poor permeability of the bacterial cell membrane and the synthesis of many antibiotic-degrading enzymes including metallo--lactamase enzymes, all contributing factors to this bacteria's high resistance.

References

[1] Shuaibu A, Machido DA. Microbiology of Otitis Media among Children Attending Clinics within Sokoto Metropolis, SOK JOU OF MED LAB SCI (SJMLS), 2017; 2(1): 6 – 11.

[2] Ngo CC, Massa HM, Thornton RB, Cripps AW. Predominant Bacteria Detected from the Middle Ear Fluid of Children Experiencing Otitis Media, a Systematic Review, PLoS One, 2016;11(3):e0150949.

[3] Nogues JC, Pérez-Losada M, Preciado D. Review of otitis media microbiome studies: what do they tell us? Laryngoscope Investigative Otolaryngology, 2020;5 (5):936–940.

[4] 4.Thornton RB, Hakansson A, Hood DW, Nokso-Koivisto J, Preciado D, Riesbeck K, et al. Panel 7 – pathogenesis of otitis media – a review of the literature between 2015 and 2019, Int J Pediatr Otorhinolaryngol, 2020;130(Suppl 1):109838.

[5] 5.Uzun L , Dal T , Kalcıoğlu M T , Yürek M , Açıkgöz Z C , Durmaz R. Antimicrobial Activity of Garlic Derivatives on Common Causative Microorganisms of the External Ear Canal and Chronic Middle Ear Infections, Turk Arch Otorhinolaryngol, 2019; 57(4): 161-5.

[6] Rashad FF, Obaid SS, Al-kadhi NA. Association of Multidrug Resistance With Biofilm Formation in *Pseudomonas aeruginosa* Isolated from Clinical Samples in Kirkuk City, NTU Journal of Pure Sciences, 2022;1(4):10-19.

[7] Mohammed SA, Tawfeeq AA, Noraldin MY. Identification and antibiotics Sensitivity of Secondary Bacterial Infection in COVID-19 (SARS-CoV-2) Pneumonia patients in Kirkuk/Iraq, NTU Journal of Pure Sciences, 2023;2(1).

[8] Imran M, Das KR, Naik MM. Co-selection of multi-antibiotic resistance in bacterial pathogens in metal and microplastic contaminated environments: An emerging health threat, Chemosphere, 2019 Jan 1;215:846-57.

[9] Aboelseoud H, Ismael E, Moustafa GZ, Badawy EM. Hygienic studies on biofilms in drinking water systems in poultry farms: isolation, molecular identification and antibiotic sensitivity, J Anim. Health Prod., 2021;9(4):443-54.

[10] Farahi RM, Ali AA, Gharavi S. Characterization of gyrA and parC mutations in ciprofloxacin-resistant *Pseudomonas aeruginosa* isolates from Tehran hospitals in Iran, Iran Jou. of Mic., 2018; 10(4):242.

[11] Flamerz RA, Obid SS, Jasim WM. Study the Effect of Biofilm Production on Antibiotic Resistance in *Proteus mirabilis* Isolated from Clinical Samples in Kirkuk City, NTU Journal of Pure Sciences, 2023 Apr 2;2(1).

[12] Vazquez-Muñoz R, Meza-Villezcas A, Fournier PG, Soria-Castro E, Juarez-Moreno K, Gallego-Hernández AL, et al. Enhancement of antibiotics antimicrobial activity due to the silver nanoparticles impact on the cell membrane, PloS one, 2019 Nov 8;14(11):e0224904.

[13] 13.Nanayakkara AK, Boucher HW, Fowler Jr VG, Jezek A, Outterson K, Greenberg DE. Antibiotic resistance in the patient with cancer: Escalating challenges and paths forward, CA: a cancer journal for clinicians, 2021 Nov;71(6):488-504.

[14] Jawad RS, Younus AH, Abbas HH, Shihab A, Jawad AR, Al Muski N. Disinfection of Drinking Water from *Escherichia coli* and *Pseudomonas aeruginosa* by Using Silver Nanoparticles, In Mat. Sci. Forum, 2020; 1002: 478-488.

[15] Çetin YS, Mollamehmetoğlu SO, Düzenli U, Turan M, Bozan N. Treatment of multi-drug resistant microorganisms in chronic suppurative otitis media, B-ENT., 2022;18(1):44-51.

[16] Musa MD. Molecular Characterization of Virulence and Antibiogram profile of *Pseudomonas aeruginosa* Isolated from Chronic Suppurative Otitis Media Patients, University of Thi-Qar Jou. of Sci (UT sci)., 2021; 8(1).

[17] Hailu D, Mekonnen D, Derbie A, Mulu W, Abera B. Pathogenic bacteria profile and antimicrobial susceptibility patterns of ear infection at Bahir Dar Regional Health Research Laboratory Center, Ethiopia, Springer Plus, 2016;5(1):466.

[18] Fadhel Z A, Hamim S S. The Frequency and Sensitivity Pattern of *Pseudomonas aeruginosa* among Otitis Media patients in Nasiriyah City, University of Thi-Qar J., 2019;14 (4):22.

[19] Jreemich SK. Isolation of Some Bacteria from Chronic Otitis Media, Al-Qadisiyah Medical Journal, 2014;10(18):159-163.

[20] Xu J, Du Q, Shu Y, Ji J, Dai C. Bacteriological profile of chronic suppurative otitis media and antibiotic susceptibility in a tertiary care hospital in Shanghai, China, Ear, Nose & Throat Journal, 2021 ;100(9): NP391-NP396.

[21] 21.Wayne PA. Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing: 20th informational supplement, CLSI document, M100-S20, 2010.

[22] Kumar R , Singh G. Study of Bacterial Pathogens and Antibiotic Sensitivity Pattern of Ear Infections in Patients with Chronic Suppurative Otitis Media Attending a Tertiary Care Hospital in Panipat, JMSH., 2019; 5(02): 19-23.

[23] Abdullah MM , Mehdi WM. Isolation and Identification of Bacteria from The Middle Ear, Nose, Pharynx, Phenotypic and Investigation of Biofilm Formation in Isolated Bacteria, Egypt. Acad J Biolog. Sci., 2022;14(2):23.237.

[24] Mohammed R Q , Abdullah PB. Infection with acute otitis media caused by *pseudomonas aeruginosa* (MDR) and *staphylococcus aureus* (MRSA), Biochem. Cell. Arch., 2020;20:(1): 905-908.

[25] Ahmad M, Hassan M, Khalid A, Tariq I, Asad MHH, Samad A, et al. Prevalence of extended spectrum β-lactamase and antimicrobial susceptibility pattern of clinical isolates of *Pseudomonas* from patients of Khyber Pakhtunkhwa, Pakistan, Bio .Med .res. int., 2016; 6068429.

[26] 26. Abdelrahman DN, Taha AA, Dafaallah MM, Mohammed AA, El Hussein ARM, Hashim AI, et al. β-lactamases (bla TEM, bla SHV, bla CTXM-1, bla VEB, bla OXA-1) and class C β-lactamases gene frequency in *Pseudomonas aeruginosa* isolated from various clinical specimens in Khartoum State, Sudan: a cross sectional study, 2020 Jul 27;9:774.

[27] Gajdács M, Bátori Z, Ábrók M, Lázár A, Burián K. Characterization of resistance in gram-negative urinary isolates using existing and novel indicators of clinical relevance: A 10-year data analysis, Life, 2020; 10:16.

[28] Abidi SH, Sherwani SK, Siddiqui TR, Bashir A, Kazmi SU. Drug resistance profile and biofilm forming potential of *Pseudomonas aeruginosa* isolated from contact lenses in Karachi-Pakistan.BMC Opht ., 2013; 13: 57.

[29] Mittal R, Yan D, Liu X Z. *Pseudomonas aeruginosa* activates PKC-alpha to invade middle ear epithelial cells, Fro.in microbiology, 2016;7:255.

[30] Bunyan IA, Hadi OM, Al-Mansoori HAK. Molecular detection of Metallo-beta lactamase producing *Pseudomonas aeruginosa* isolated from different sites of infection, J. Pharm. Sci. & Res., 2018;10(5): 1072-1078.