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Isolation and Antibiotic Sensitivity Testing of *Pseudomonas aeruginosa* Isolates from Patients with Chronic Suppurative Otitis Media in Baghdad

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Abstract

Otitis media is one of the most common types of ear infections, plus bacteria are to blame. *Pseudomonas aeruginosa* is the most frequently diagnosed pathogen causing this infection. *P. aeruginosa* has virulence factors that lead to damage to the middle ear mucosa. This study was conducted to identify *Pseudomonas aeruginosa* causing chronic suppurative otitis media and to determine its antibiotic sensitivity. A total of (100) ear swabs were collected from patients suffering from otitis media attending the ENT outpatient clinic at Baghdad Teaching Hospital from July 2022 till the end of December 2022. Age range was (18-75) years. Identification of bacteria and biochemical testing and antibiotic sensitivity test of the bacteria spp. were detection of 18 antibiotics for different types were carried out using the VITEK 2 automated system. The study showed the results as follows: Eighty positive cases, distributed among 32 (40%) males and 48 (60%) females. About 25% of patients are between 48-57 years old and 58-67 years old, respectively. A total of 55 patients (68.75%) were from urban areas, and 25 patients (31.25%) were from rural areas .With regard to the antibiotic susceptibility profile, the data showed that the most effective antibiotics were colistin(77%) Ceftazidime(55%) ,Cefepime(45%), Imipenem (42%), highest resistance percentages of strains to tested antibiotics was for Ceftoriaxone , Cefpodoxime (100%) and the lowest resistant percentages was (22.5%) for colistin Females were more likely to develop otitis media, and the prevalence appeared to be higher among the elderly. *Pseudomonas aeruginosa* represents the most implicated bacterial pathogen associated with this infection. colistin is the most effective antibiotic and can be considered as the drug of choice against *P. aeruginosa*.

Keywords: Otitis media, ear infection, *Pseudomonas aeruginosa*, antibiotic sensitivity of ear infection.

1.Introduction

Pseudomonas aeruginosa is one of the important gram-negative bacteria. It is found in waters, soil, plants, animals, hospitals, and on the skin of natural persons (1,2). *Pseudomonas aeruginosa* is an opportunistic pathogen that causes various infections such as chronic-suppurative otitis media (CSOM), nosocomial bacterial infection, and urinary tract infection (3,4).An infection of chronic suppurative otitis media (ESOM) is defined as chronic middle ear and mastoid cavity inflammation characterized by perforated tympanic membrane and persistence of ear dis charge(5).Multidrug resistance P. aeruginosa (MDRPA) is a rapidly developing and growing problem in recent years. It involves a resistance toward less than three drugs of different categories (6,7) This study was carried out and aimed to identify the main bacterial causative agents of otitis media, as well as to determine the antibiotic susceptibility pattern of the isolates, in order to define the most appropriate antibiotics used in the treatment of such infections.

2. Materials andMethods

2.1 Samples collection:

A total of 100 ear swabs were collected from patients with otitis media attending ENT department at Baghdad Teaching Hospital from July 2022 to the end of December 2022. Age ranged from 18-75 years.

2.2 Inclusion criteria

Patients with otitis media only without other microbial infection.

2.3 Exclusion criteria

Patients on antibiotics within the last 3 days, patients with discharging ears of less than 3 weeks duration, and immunocompromised patients with chronic diseases, organ transplants, or immunosuppressive drugs.

2.4 Clinical examination

A complete history and physical examination were done for each patient. The current study identified aural discharge as the primary clinical feature.

2.5 Bacterial Isolation

All clinical samples were cultured by streaking on Blood agar, MacConkey agar, and Cetrimide agar, and incubated for 24 hours at 37°C. This isolates P. aeruginosa growth on Cetrimide agar, which is the selective media for bacterial.

2.6 Bacterial Identification

The colony morphology on Blood agar, MacConkey agar, and Cetrimide agar was dependent on the colony shape, texture, color, and edges. We examined the macroscopic characteristics of a gram-stained slide under a light microscope, and paying particular attention to the shape and arrangement of the cells. We conducted traditional biochemical tests and utilized the VITEK 2 compact system to ensure thorough identification.

2.7 Antibiotic susceptibility test

We tested the susceptibility of P. aeruginosa isolates to antibiotics such as Amikacin, Cefepime, Ceftazidime, Cefpodxime, Ceftoriaxone, Colistin, Gentamicin, Imipenem,

Meropenem, Piperacilin, Ticarcillin, Clavulanic Acid, Tobramycin, moxifloxacin, norfloxacin, ofloxacin, levofloxacin, and ciprofloxacin, following the manufacturer's instructions.

2.8 Statistical analysis

Statistical inference for the Social Science SPSS (version 21, GraphPad Software, San Diego, California, USA) program was used for data entry and analysis The statistical significance level was established at a *P* value ≤ 0.05.

3. Results

This study included 80 out of the 100 total number of *P. aeruginosa* isolates found to be positive. *P. aeruginosa* has been found to be the most implicated pathogen with the higher number of isolates. The age groups of the patients were (38-47), (48-57), and (58-67) years with percentages of (22.5%, 25%, and 25%), respectively, which was found to be a highly statistically significant association ($P \leq 0.01$). **Table** 1 displays the results.

Table 1. Distribution of patients according to age groups.

Age groups (years)	Total No.	Positive No. (%)	Negative No. (%)
18-27	11 (11%)	5 (6.25%)	6 (30%)
28-37	14 (14%)	12 (15%)	2 (10%)
38-47	20 (20%)	18 (22.5%)	2 (10%)
48-57	23 (23%)	20 (25%)	3 (15%)
58-67	22 (22%)	20 (25%)	2 (10%)
68-77	10 (10%)	5 (6.25%)	5 (25%)
Total	100	80	20
P value	---	0.0001 **	0.0294 *

* ($P \leq 0.05$), ** ($P \leq 0.01$)

In terms of gender, it was found that the 80 positive ear swabs belonged to 32 (40%) males and 48 (60%) females, with non-significant differences ($P \leq 0.05$). The results are displayed in **Table 2**.

Table 2. Distribution of patients according to the gendr

Gender	Total No. (%)	Positive No. (%)	Negative No. (%)
Male	40 (40%)	32 (40%)	8 (40%)
Female	60 (60%)	48 (60%)	12 (60%)
Total	100	80	20
P value	0.0455 *	0.0736	0.361

* ($P \leq 0.05$)

Data revealed that the highest percentage of Otitis media was recorded among urban patients 55 (68.75%) than in rural patients 25 (31.25%), with a highly statistically significant difference ($P \leq 0.01$). The results are displayed in **Table** 3.

Table 3. Distribution results of sample according to their residency

Residency	Total No. (%)	Positive No. (%)	Negative No. (%)
Urban	70 (70%)	55 (68.75%)	15 (75%)
Rural	30 (30%)	25 (31.25%)	5 (25%)
Total	100	80	20
***P* value**	0.0001 **	0.0008 **	0.0025 **

** ($P \leq 0.01$)

As for infection site, however, the findings of the current study exhibited that the ear swabs from the left ear revealed a higher number of positive swabs (62.5%%), followed by ear swabs from the right ear (37.5%), with a significant difference ($P < 0.05$). The results are shown in **Table 4.**

Table 4. Distribution of patients according to site of infection

Site of infection	Total No. (%)	Positive No. (%)	Negative No. (%)
Right ear	35 (35%)	30 (37.5%)	5 (25%)
Left ear	65 (65%)	50 (62.5%)	15 (75%)
Total	100	80	20
***P* value**	0.0027 **	0.0253 *	0.0253 *

* ($P \leq 0.05$), **($P \leq 0.01$)

The seasonal infection: As shown in Table 5, the results of the current study indicate that this infection appears to be higher in November and December.

Table 5. Distribution of patients according to seasons

The month	Total no	Positive no	Negative no
July	5	3	2
August	8	4	4
September	10	8	2
October	12	10	2
November	26	20	6
December	39	35	4
The total	100	80	20

According to the results of **Table (6) and Figs. (1,2),** the antibiotic susceptibility test was performed on 80 *P.aeruginosa* isolates by using the VITEK 2 compact system, which has the ability to determine multidrug resistance of the 18 antibiotics used against the tested isolate according to the manufacturer's instructions (BioMérieux). All *Pseudomonas aeruginosa* isolates are highly resistant (90–100%) to cefpodxime, ceftoriaxone, moxifloxacin, norfloxacin, and levofloxacin. The VITEK 2 compact system had a 98% probability.

Table 6. Antibiotic susceptibility pattern of the positive isolates

Antibiotics	Resistance	Sensitive	Intermediate	*P* value
Ticarcillin	62 (77.5%)	18 (22.5%)	0 (0)	0.0001 **
Clavulanic acid	62 (77.5%)	18 (22.5%)	0 (0)	0.0001 **
Piperacillin	72 (90%)	8 (10%)	0 (0)	0.0001 **
Cefpodoxime	80 (100%)	0 (0)	0 (0)	0.0001 **
Ceftazidime	36 (45%)	54 (55%)	0 (0)	0.0001 **
Ceftoriaxone	80 (100%)	0 (0)	0 (0)	0.0001 **
Cefepime	54 (55%)	36 (45%)	0 (0)	0.0001 **
Imipenem	45 (56.25%)	35 (43.75%)	0 (0)	0.0001 **
Meropenem	45 (56.25%)	35 (43.75%)	0 (0)	0.0001 **
Amikacin	62 (77.5%)	18 (22.5%)	0 (0)	0.0001 **
Gentamicin	62 (77.5%)	18 (22.5%)	0 (0)	0.0001 **
Tobramycin	62 (77.5%)	18 (22.5%)	0 (0)	0.0001 **
Ciproflaxacin	62 (77.5%)	18 (22.5%)	0 (0)	0.0001 **
Levofloxacin	72 (90%)	8(10%)	0 (0)	0.0001 **
Colistin	18 (22.5%)	62 (77.5%)	0 (0)	0.0001 **
Moxifloxacin	72(90%)	8 (10%)	0 (0)	0.0001 **
Norfloxacim	72(90%)	8 (10%)	0 (0)	0.0001 **
Ofloxacin	71(88.75%)	9 (11.25%)	0 (0)	0.0001 **
***P* value**	**0.0001 ** **	**0.0001 ** **	**1.00**	**---**
** (*P* ≤ 0.01)**				

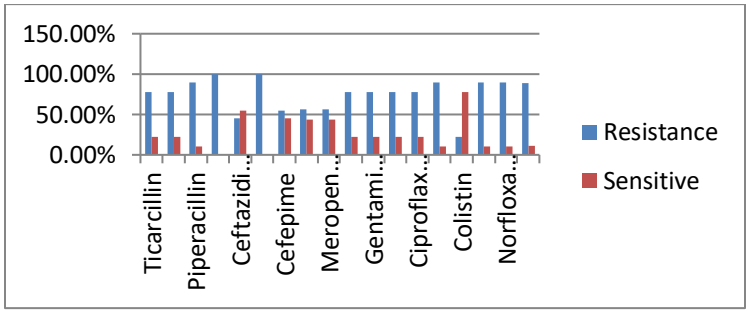

Figure 1. Antibiotic susceptibility pattern of the positive isolates

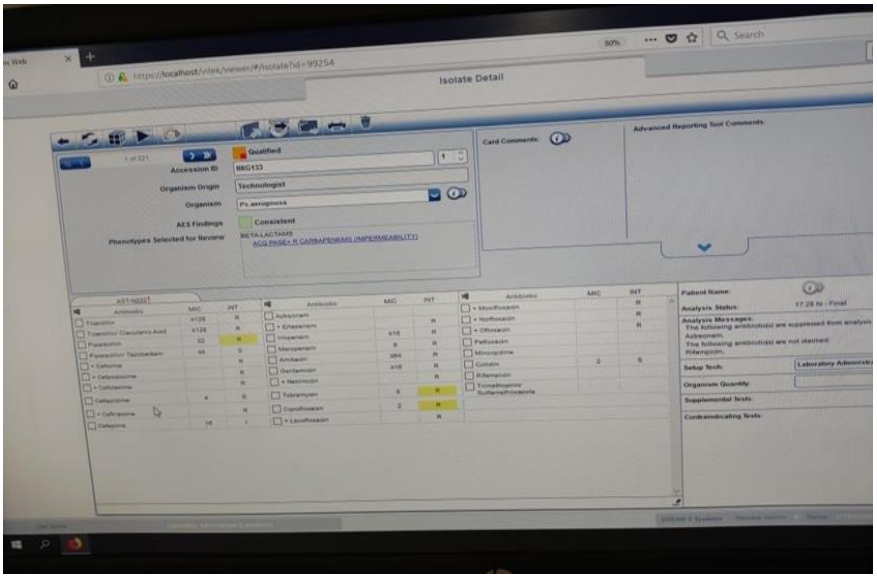

Figure 2. Antibiotic susceptibility test by using VITEK 2 compact system.

4. Discussion

The current study demonstrated that 80% of patients with otitis media had a positive *Pseudomonas aeruginosa,* and this result agreed with (1). Additionally, many references should be included. Write that this bacteria was more common than others, Data analysis revealed that patients in the first age group (18-27) years recorded a lower prevalence of positive cases, whereas patients in the third age group (38-47) years showed a higher prevalence. These findings, however, agree with the results of two recent studies done in (2020) and (2021) (7,8) ,and disagree with the results of a study done in (2017) demonstrated that a high prevalence was recorded among patients under 30 years old (6). Many factors, including the impaired immune status of elderly people, to an increase in the microbial infection rate as age increases (9).

Regarding sex of the patients, data revealed that incidence among females was higher than males, which in turn was compatible with other studies (10,11) and incompatible with the outcomes of a study carried out in (2017) (6). Females were at higher risk of infection with *P. aeruginosa* due to sex hormones as well as impaired immune status. Generally, testosterone has an immunosuppressive effect, while estrogen has an immune-enhancing effect on the immune system. Researchers have found that estrogen manages the immune system by stopping the negative selection of high affinity auto-reactive B cells, changing the way B cells work, and starting the Th2 response. Progesterone suppresses specific components of the immune system and natural killer (NK) cells activity, whereas it has a mainly positive influence on other nonspecific components. Elevated cortisol suppresses your immune system by decreasing white blood cell production. Without white blood cells on the hunt for germs, your response to an infection is much slower.

As for the site of infection, it was demonstrated that ear swabs from the left ear revealed a higher number of positive swabs than from the right ear, which was incompatible with the findings of a recent study done in 2020 showing the opposite (12).

The coldness of November, December, January, and February contributes to the prevalence of respiratory diseases during these cold months. The current study also determined the seasonal incidence. The current study indicates a higher incidence of this infection in November, in line with the findings of previous studies (13,16). In autumn seasons, especially in November, the nasal allergy is considered a major risk factor associated with the occurrence of this infection. Moreover, mast cells secrete inflammatory mediators in response to an allergen, which could lead to mucosal damage and increased secretions (13).

In our current study, we found that the percentage of infection with these bacteria is higher in the city than in the countryside, due to the indiscriminate use of antibiotics and lack of interest in health due to difficult life conditions and stressful work. In the current study, the rate of patients from urban areas was higher than in rural areas, which is compatible with another study done in Iraq that revealed that the prevalence of patients from Al-Hawija city (urban) was higher than rural areas around it (14)..

P. aeruginosa uses intrinsic, acquired, or adaptive resistance as its defense mechanism against antibiotics. In the intrinsic phase, the outer membrane is not permeable, efflux pumps are expressed that push antibiotics out of cells, and enzymes that destroy antibiotics are made. A horizontal shift of resistance genes or mutational changes might achieve the acquired phase (15).

The isolates' antibiotic susceptibility patterns revealed a high resistance to ciprofloxacin and norofloxacin, in line with other studies that showed resistance rates of ciprofloxacin at 69% and norofloxacin at 81% (16). Two studies done in the south of Iraq in 2016 and 2019 exhibited that the resistance rate of ciprofloxacin and norofloxacin was lower.

This work detected a higher gentamycin resistance rate. These findings agree with other studies demonstrating a higher resistance pattern related to this antibiotic (16,17) and disagree with others (18,19) .This study also detected high resistance to tobramycin, in line with other studies (20,21). However, for the amikacin, high resistance was also shown in (22), and lower resistance (incompatible findings) were demonstrated in (7, 29). For piperacillin, the resistance rate observed was high, and this corresponds with (23,30) and not with (24).

This study shows that resistance toward meropenem and imipenem was similar (56%), which is consistent with (23,25) and disagrees with (26,27).Resistance can change for many reasons, such as the ability to change how permeable the membrane is, making beta-lactamase enzymes, biofilm formation, and R-resistance plasmids that have different antibiotic resistance genes (28,31).

5. Conclusion

The present study identified *Pseudomonas aeruginosa* as the common cause of CSOM. P. aeruginosa was resistant to a variety of commonly used antibiotics when isolated from patients with chronic suppurative otitis media. Females were at higher risk of otitis media, with a prevalence rate that appears to be higher among the elderly. *Pseudomonas aeruginosa* is the most commonly implicated bacterial pathogen associated with this infection.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Ethical Clearance

The study was conducted in accordance with the ethical principles. It was done with patients' verbal and analytical approval before the sample was taken. The study protocol and the subject information and the consent form were reviewed and approved by Baghdad University, the College of Medicine a local ethics committee according to the document number (Ref. No. 026, Date 5/6/2023) to get this approval.

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