



**ANTIBIOTIC SUSCEPTIBILITY OF PSEUDOMONAS AERUGINOSA EYE INFECTION
AMONG IRAQI PATIENTS**

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ABSTRACT

Introduction: The most severe form of these infection is bacterial keratitis that if not treat correctly, can lead to scarring and blindness, *P. aeruginosa* can commonly infect contact lens consumers causing corneal ulceration as it contaminates moist areas the areas of the eye that are commonly infected are the conjunctiva, lid and cornea.

Method: Through (100) eye swabs, from 100 (60 males and 40 females) were included in this study by taking eye swabs. Twenty cases (20%) with *Pseudomonas aeruginosa* positive isolates were found in patients who consult various hospitals in Baghdad city between March 5 and May 7, 2022. twenty (20) isolates of *Pseudomonas aeruginosa* were evaluated for their antibiotic susceptibility against eleven different antibiotics using Kirby-Bauer disc diffusion methods revealed varying degrees of sensitivity.

Result: *P. aeruginosa* that the most common pathogen causes of eye infections among male children below 20 years. isolates showed multidrugs resistance belong to Ampicillin, Piperacillin, Chloramphenicol and Tetracycline, whereas the most isolates were sensitive to Meropenem, Gentamicin, Ciprofloxacin, Amikacin, Tobramycin and Ceftazidime

Conclusion: -eye infection with *pseudomonas aeruginosa* was the common treatable eye disease

KEYWORDS:- Eye infection, *Pseudomonas eauroginosa*, Antibiotic susceptibility, Meropenem, Cetazedim, Baghdad city hospitals

INTRODUCTION

Infections of the eyes are usually linked with microbial contamination of contact lenses. While eyes can obtain infections from bacteria, fungi, viruses and other pathogenic agents. The most severe form of these infection is bacterial keratitis that if not treat correctly, can lead to scarring and blindness (Willcox, 2007). Eye disease, named as ulcerative keratitis has been considered as the most critical bacterial disease of human cornea and causes a severe inflammatory response to bacterial infection of



cornea (Lyczak *et al.*, 2000). *P. aeruginosa* can commonly infect contact lens consumers causing corneal ulceration as it contaminates moist areas (Stewart *et al.*, 2011). Pathogenic microbes cause ocular disease due to virulence and host's reduced resistance because of the factors like personal hygiene, living conditions, socioeconomic status, decrease immune status, where's the areas of the eye that are commonly infected are the conjunctiva, lid and cornea (Ubani, 2009 and Ramesh *et al.* 2010).

MATERIALS AND METHODS

Patients: One hundred samples were collected from patients including (60 males and 40 females) with age ranged from less than 65 years, who suffered from eye infections diagnosed by ophthalmologists. that presenting to five hospitals of Baghdad city during the period from 5 March 2022 to 7 May 2022.

Specimen collection:

Cotton swabs and corneal scrapes were used to collect eye samples from the exterior ocular surface. These samples were placed directly onto transport medium, then incubated overnight at 37 C. (Mims *et al.*, 2008). Three different culture media, including Blood agar and MacConkey's, Cetrimide agar (Morello *et al.*, 2006). were inoculated with each swab after it had been taken directly by transport medium. The morphological properties analysis starting with Gram stain and appearance under a light microscope (Gram reaction, arrangements, and form), colonies that grow on the selected media were further identified (Collee *et al.*, 1996).

-A questionnaire was used to document each case's history.

Statistical analysis: SPSS (V-15) statistical software and Excel 2007 data are used in the (P5) computer. Table designs (number, percentage, mean, and standard error) and graphic presentations like figures were included in the descriptive statistics. While Chisquare, t-test, and Z-test are used as inferential statistics (McCall, 1980).

RESULTS AND DISCUSSION

Patients with eye infections who presented as outpatients in five hospitals at Baghdad City provided a total of 100 samples. Cellular features, cultural morphology, and biochemical tests were employed as the primary categorization phenotypic tests for the isolation and identification of isolated bacteria. These tests were selected as conventional tests by the Mini API 20NE method and Vitek2 system. To identify the isolated microorganisms, these assays were chosen as standard procedures (Collee *et al.*, 1996).



Figure (1): Mini API 20NE system showed positive result for *P. aeruginosa* strain.

20 miniature biochemical tests from left to right :+veNO₃, -veTRP, -veGLU, -veADH , -ve URE, -ve ESC, +veGEL, -ve PNPG, +ve GLU, -ve ARA, - ve MNE, +veMAN , -ve NAG, -ve MAL, +veGNT , +veCAP , +ve ADI, +ve MLT, +ve CIT, -vePAC .

Antibiotics susceptibility profile for *P. aeruginosa* isolates:

of the twenty (20) isolates of *P. aeruginosa*. susceptibility to ten (10) antibiotics was examined By using Kirby-Bauer disc diffusion methods,. antibiotic susceptibility was screened using agar screening techniques (CLSI, 2011) On Muller Hinton agar,. The antibiotics chosen to treat *P. aeruginosa* are listed in Table (1), and the VITEK-2 system was used to test the medications' susceptibility.

1- Meropenem:

is an effective antibiotic that is used to treat wide spectrum of infections, Similar to imipenem and ertapenem, it is a β -lactam and a member of the carbapenem subgroup. *P. aeruginosa* isolates demonstrated greater susceptibility to 19 (95%) and 1 (5%) intermediate , with high significant differences ($P < 0.01$) found among other antibiotics. These findings support Pour et al(2011) , that *P. aeruginosa* isolates were more likely to be susceptible to imipenem(96%) (which is comparable to meropenem) than to be resistant to it (4%). While Goudarzi et al. (2011) stated that 57 (100%) of patients were imipenem sensitive, this information was the same of our current study. While in Jácome et al(2012) study the finding disagree with our study in which that activity of Meropenem (62.3%) and imipenem (63.9%) drugs against *P. aeruginosa*.

- Pires *et al.*,(2009) observed that the most active drug against *P. aeruginosa* was meropenem(79.3%), imipenem(81.8%)and these results nearly to current study.

2. Ceftazidime:

Ceftazidime, a β -lactam antibiotic originally derived from the fungus Acremonium, formerly known as cephalosporium, was the third generation of cephalosporin. With antipseudomonal activity, *P. aeruginosa* isolates demonstrated sensitive to ceftazidime 14 (70%) and resist 6 (30%) with no



statistically significant differences ($P > 0.05$). These results are comparable to those reported by Long *et al* Long *et al.* (2014) who noted *P. aeruginosa* as being largely susceptible to ceftazidime (75%) and Jácome *et al.* (2012) who noted 13 (44.8%) of the 29 isolates as being resistant to ceftazidime. According to Pour *et al.* (2011), *P. aeruginosa* is extremely susceptible to ceftazidime 100 (100%) and these findings are relevant to the current study. Prashanth *et al.* (2010) reported Most of the 39 isolates (100%) from the Hyderabad hospital were sensitive to routinely used antibiotics such as ceftazidime. These findings agree with those of our study to a lower extent.

3 Gentamicin:

antibiotic belong to aminoglycoside group used to treat various bacterial infections, especially those brought on by Gram-negative organisms Moulds *et al.* (2010). It is made up of a combination of related Gentamicin components and fractions. Gentamicin was highly effective in the current study with 19 (95% efficacy) and 1 (5% intermediate) showing highly significant differences ($P < 0.01$) observed.

Table (1): Susceptibility testing profile of *P. aeruginosa* isolates from eye infections

Antibiotics		N	%	P-VALUE
Meropenem	Sensitive	19	95%	P=0.00 HS ($P < 0.01$)
	Intermediate	1	5%	
	Total	20	100%	
Ceftazidime	Sensitive	14	70%	P=0.115 NS ($P > 0.05$)
	Resist	6	30%	
	Total	20	100%	
Gentamicin	Sensitive	19	95%	P=0.00 HS ($P < 0.01$)
	Resist	1	5%	
	Total	20	100%	
Amikacin	Sensitive	17	85%	P=0.003 HS ($P < 0.01$)
	Resist	3	15%	
	Total	20	100%	
Tobramycin	Sensitive	16	80%	P=0.012 S ($P < 0.05$)
	Resist	4	20%	
	Total	20	100%	
Ciprofloxacin	Sensitive	19	95%	P=0.00 HS ($P < 0.01$)
	Resist	1	5%	
	Total	20	100%	

Goudarzi *et al.*, (2011) reported that *P. aeruginosa* isolates of the study were sensitive to Gentamicin 55(96.5) versus resist 2(3.5%) this results nearly to present study. On the other hand Pour *et al.*, (2011) study referred that Gentamicin was sensitive (93%) versus resist (7%) these results agree with current study. While Flayyih *et al.*, (2013), were found 100(100%), and all these isolates were sensitive to



Gentamicin, so these results a approach to results of current study. Patel *et al.*, (2009) were showed the susceptibility of isolates were reduced to Gentamicin (76.4% to 62.3% from 329 total specimen). These results differ from the present study results. Prashanth *et al.*, (2010) were reported most of the isolates 39(100%) from Hyderabad hospital were resistant to commonly tested antibiotics such as Gentamicin and also these results disagree with current study table (1).

4- Amikacin:

aminoglycoside that is used to treat various bacterial infections. Amikacin is most frequently used to treat severe, hospital-acquired infections caused by multidrug resistant gram negative bacteria like *P. aeruginosa*. It works by attaching to the bacterial 30S ribosomal subunit, which results in misreading of mRNA and prevents the bacterium from synthesizing proteins necessary for its growth (WHO,2013).

The results of the current study showed that 17(85%) were sensitive to Amikacin and resist 3(15%) with highly significant differences ($P<0.01$) observed among other antibiotics. These results agree with Bharathi *et al.*, (2010) who reported that the Gram negative organisms were susceptible in highest percentage to Amikacin 721 (93.51%). Patel *et al.*, (2009) observed the susceptibility of *P. aeruginosa* isolates were reduced to Amikacin (98.4% to 94.1% of 329 total specimen) and Tesfaye *et al.*,(2013) who referred that gram negative bacteria which were highly sensitive towards Amikacin 30(96.8%) and these results nearly to current result. While Pour *et al.*,(2011) who reported that percentage of *P. aeruginosa* isolates were sensitive to Amikacin and (97%) opposed to resist (3%)and also these find approach to present study .Flayyih *et al.*,(2013) study, found 100(100%) of these isolates were sensitive to amikacin,these results differ from result of current study. Prashanth *et al.*,(2010) reported most of the isolates 39(100%)from Hyderabad hospital were resistant to commonly tested antibiotics such as Amikacin this results disagree with study. Table (1)

5 Tobramycin:

aminoglycoside produced from *Streptomyces tenebrarius* that is used to treat various Gram-negative bacterial infections. *Pseudomonas* species are particularly susceptible to its effects. Tobramycin blocks the formation of the 70S complex by attaching to a specific location on the bacterial 30S and 50S ribosome. Because of this, mRNA cannot be converted into protein, which causes cell death. In this study *P. aeruginosa* isolates were sensitive to tobramycin antibiotic 16(80%) and resist 4(20%) and observed significant differences ($P<0.05$), these finding approach to Long *et al.*,(2014) referred to *P.aeuroginosa* relatively was sensitive to tobramycin (75%), while Patel *et al.*,(2009) showed that susceptibility was reduced in tobramycin from (83.6%) to (64.1%) of 329 total isolates. This result differs from current result, table (1).

6 Ciprofloxacin:

is a quinolone medication class synthetic chemotherapeutic antibiotic. It is a fluroquinolone antibacterial of the second generation. In particular, ciprofloxacin, which is frequently used to treat *P.*



aeruginosa infections, inhibits bacterial infections by interfering with the enzymes that cause DNA to unwind after being duplicated, which halts the synthesis of DNA and protein (Nelson *et al.*, 2007), specially ciprofloxacin which commonly used antibiotic against *P.aeruginosa* (Poole, 2011) with high serum level while the first generation Nalidixic acid of minimum serum level, not so effective against *p. aeruginosa*, (Heidelbaugh and Holmstrom, 2013).

The findings revealed a significant differences ($P < 0.01$), ciprofloxacin sensitivity of 19 (95%), and resistance of 5%.

These findings are in line with those of Pour *et al.*, (2011) and Goudarzi *et al.*, (2011) who found that ciprofloxacin was very sensitive in 52 and 57 percent of cases, respectively. While Tesfaye *et al.* (2013), Bharathi *et al.* (2010), and Long *et al.* (2014) found that *P. aeruginosa* was relatively sensitive to ciprofloxacin (83.3%), 26 (83.9%), and 668 (86.64% from total 771) respectively, these results nearly match the current study. As opposed to the current result, Patel *et al.* (2009) reported that of the 329 total isolates, the susceptibility to ciprofloxacin had decreased from 76.3 to 45.9%. which differ from our result.

While Prashanth *et al.*, (2010) reported most of the isolates 39 (100%) from Hyderabad hospital were resistant to commonly tested antibiotics such as ciprofloxacin and these results disagree with the results of present study.

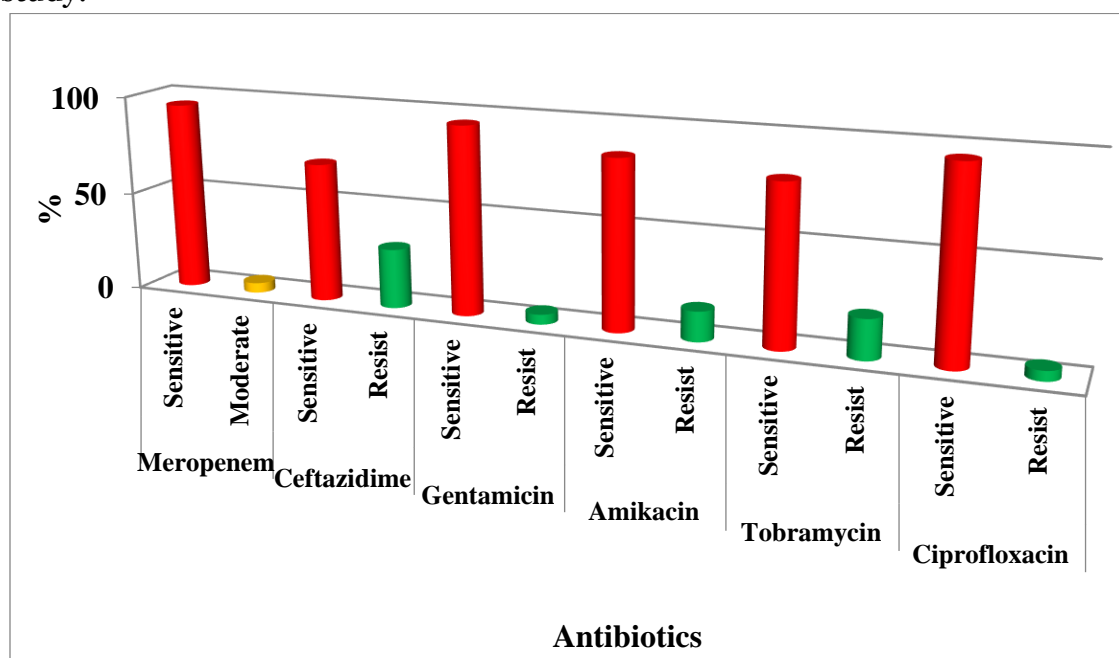


Figure (2): Susceptibility testing profile of *P. aeruginosa* isolated from eye infections.

7 Ampicillin:

Is an antibiotic effective in treating various bacterial infections. It is a beta-lactam antibiotic that can penetrate both some gram positive and some gram negative bacteria. It is a member of the aminopenicillin family and the penicillin beta-lactam antibiotic subclass. It only differs from penicillin



G, or benzylpenicillin, in that it has an amino group, which makes it more effective in penetrating gram negative bacteria's outer barrier. Transpeptidase, an enzyme required by bacteria to build their walls, is inhibited permanently by ampicillin. It prevents binary fission's third and final stage of bacterial cell wall construction, which results in cell lysis and is regarded as bactericidal (Petri et al., 2011). In this study, ampicillin antibiotic percentage was resist 19(95%), intermediate 1(5%) with highly significant differences ($P < 0.01$).

Table (2): Antibiotic resistance of *P. aeruginosa* isolated from eye infections.

Antibiotics		N	%	Z-test (P-value)
Ampicillin	Resist	19	95%	P=0.00 HS ($P < 0.01$)
	intermediate	1	5%	
	Total	20	100%	
Piperacillin	Resist	19	95%	P=0.00 HS ($P < 0.01$)
	intermediate	1	5%	
	Total	20	100%	
Tetracycline	Resist	18	90%	P=0.00 HS ($P < 0.01$)
	intermediate	2	10%	
	Total	20	100%	
Chloramphenicol	Resist	19	95%	P=0.00 HS ($P < 0.01$)
	intermediate	1	5%	
	Total	20	100%	

These results are near to AL-Abidi,(2005) who found all isolates were resist to Ampicillin 12(100%),while Tesfaye *et al.*,(2013) were reported that the majority of the Gram-negative cocci was 56 (72.7%) from total 77 showed resistance to penicillin. So that Ubani,(2009) observed that all isolates 34(100%) were not sensitive to penicillin.

8- Piperacillin:

Is an extended spectrum beta-lactam antibiotic of the ureidopenicillin class (ureidopenicillins are a group of penicillin which are active against *P. aeruginosa*). It is normally used together with a beta-lactamase inhibitor, Piperacillin was tested in the study and showed that the study isolates were resist 19(95%) and only one isolate appeared as intermediate resistance 1(5%)with highly significant differences ($P < 0.01$), these results in harmony with Flayyih *et al.*, (2012) who found that the two isolates (100%) of *P. aeruginosa* were resistant to Piperacillin.

9 -Tetracyclin:

The results showed the study isolates were resist to tetracycline 18(90%) and two isolate as intermediate 2(10%) with highly significant differences ($P < 0.01$), these results nearly to AL-Abidi,(2005) who observed that *P. aeruginosa* isolates were resist to tetracycline 10(83.3%) and sensitive 2(16.6%) while



Flayyih *et al.*, (2013) reported that 100(100%) of isolates were resist to tetracycline antibiotics and these results approached to present study. Other studies Tesfaye *et al.*, (2013) showed resistance to tetracycline which about 22 (71.0%). Pour *et al.*, (2011) referred that bacterial isolates were resist to tetracycline 37(71%), and sensitive 15(29%) so that these results slightly agree with current study table (2).

10- Chloramphenicol:

Is an antibiotic effective in treating various bacterial infections. By suppressing protein synthesis, it acts as a bacteriostatic. By stifling the peptidyl transferase activity of the bacterial ribosome, it hinders protein chain elongation. *Staphylococcus aureus*, *Streptococcus pneumonia*, and *Escherichia coli* are just a few of the bacteria that can cause eye infections that can be successfully treated with chloramphenicol due to its broad range of activity. It is ineffective when used to treat *P. aeruginosa* (Fetar *et al.*, 2011 and Poole, 2011).

The result of the study isolates were resist to Chloramphenicol antibiotic 19(95%), one isolate as intermediate 1(5%) and highly significant differences ($P < 0.01$), these results nearly to Pour *et al.*, (2011) who referred that *P. aeruginosa* isolates was resist to Chloramphenicol 50(97%) and sensitive 2(3%). So that Ubani, (2009) observed that (90%) of isolates resist to Chloramphenicol, and 10% sensitive so these results was slightly agree with current study. While Prashanth *et al.*, (2010), Goudarzi *et al.*, (2011) and AL-Abidi, (2005) showed most of the *P. aeruginosa* isolates resistant to Chloramphenicol 39(100%), 57(100%) and 12(100%) respectively, these results approach to current result. Tesfaye *et al.*, (2013) referred that isolates was resist to Chloramphenicol 12(38.7%), sensitive 16(51.6%) and intermediate 3(7.9%) therefore these results disagree with present study figure (3).

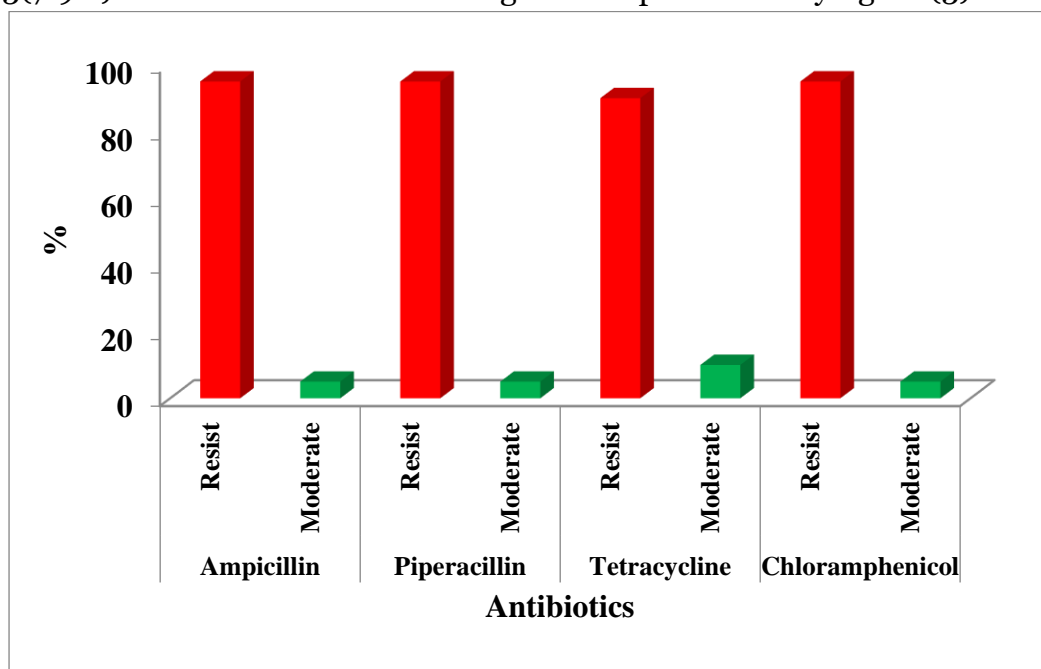


Figure (3): Antibiotic resistance of *P. aeruginosa* isolated from eye infections.



Antimicrobial susceptibility testing used VITEK-2 system:

In the present study new VITEK-2 compact system Gram negative antimicrobial susceptibility testing (AST card, biomeriux), VITEK 2 provide AST cards results according to the manufacturer's instructions for antimicrobials as susceptible, intermediate and resistance were used to determine the antibiotics susceptibility of *P. aeruginosa*, twenty (20) isolates to fifteen (15) antibiotics (Ampicillin, Piperacillin /Tazobactam, Cefazolin, Ceftazidime, Ceftriaxone, Cefepime, Imipenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin, Levofloxacin, Tigecycline, and Trimethoprim/Sulfamethoxazole).

-The results of this method showed (appendix III) that nearly to results obtained by used Kirby-Bauer disc diffusion methods. Jaafar *et al.*, (2014) reported by VITEK 2 that twenty eight *P. aeruginosa* isolates (96.5%) were resistant to Ceftriaxone, 8 (27.59%) were resistant to Ceftazidime and Gentamicin, and 7 (24.14%) resistant to Cefepime, Imipenem, Tobramycin, Amikacin, Ciprofloxacin, and Levofloxacin, so that 5 (17.24%) were resistant to meropenem. These results approach with current study. While Hussein *et al.*, (2015) showed that 16(80%) isolates were sensitive to Amikacin, 20(100%) *P. aeruginosa* isolates were resist to Ampicillin, Gentamicin and Imipenem 12(60%) and 20(100%) respectively sensitive. These results agree with the present study. So that 20(100%) of isolates resistance to Tobramycin, these results disagree with current study.

Conclusions

According to the results obtained in the study, the following conclusions have been reached at:

- 1- The study proved that *P. aeruginosa* that the most common pathogen causes of eye infections.
- 2- Conjunctivitis was the most common infection among children below 20 years.
- 3- VITEK 2 system and analytical assays API 20NE consider as an important confirmative tool for diagnosis of *P. aeruginosa* isolates.
- 4- *P. aeruginosa* isolates were present in high percent among male children patient with eye infection below 20 years age.
- 5- The antibiotics susceptibility obtained by Kirby-Bauer and VITEK-2 system methods revealed that *P. aeruginosa* isolates showed multidrugs resistance belong to Ampicillin, Piperacillin, Chloramphenicol and Tetracycline, whereas the most isolates were sensitive to Meropenem, Gentamicin, Ciprofloxacin, Amikacin, Tobramycin and Ceftazidime.

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