Sys Rev Pharm 2020;11(12):1117-1125
A multifaceted review journal in the field of pharmacy

A Review: Antimicrobial Agent for Pseudomonas aeruginosa Isolated From Iraqi Patients

[1]Saade Abdalkareem Jasim, [2]Huda Taher Feisal, Lubna [3]Abdulazeem

[1]Medical laboratory techniques department, Al-maarif University College, IRAQ
[2]Biology Department, College of Educatiom for Pure Sciences, Thi-Qar University, IRAQ
[3]DNA Research Center, University of Babylon, IRAQ
Corresponding Author: saadee198814@gmail.com

ABSTRACT

Pseudomonas aeruginosa This is study aimed to review previous studies that explain the causes of multiple resistance to these bacteria, and they are also considered Nosocomail bacteria. These bacteria most often infect immunocompromised people, and treatment is often difficult and complicated for those infected due to multiple resistance. This review also demonstrated the benefit of using plant extracts and aromatic oils as alternatives to antibiotics as an inhibitor against P. aeruginosa. During previous studies, it was shown that most of the isolates were distributed among burn infections, wounds, urinary and respiratory tract infections, sputum, and blood poisoning. In addition, this review focused on virulence genes and their major role in bacterial resistance to antibiotics, as well as identifying the main P. aeruginosa genes to be used in the speed of diagnosis. Among the most important of these virulence factors is biofilm formation, β-lactamase production, Pyocyanins, and other enzymes. This review also identified the differences between clinical and environmental isolates and the extent of the effect of random use of antibiotics and the modifications that occurred to bacteria that made them resistant to these antibiotics.

Keywords: P. aeruginosa, Antimicrobial agents, Virulence factors, MIC, β-lactamase.

Correspondence:
Saade Abdalkareem Jasim
1Medical laboratory techniques department, Al-maarif University College, IRAQ
*Corresponding author: Saade Abdalkareem Jasim email-address:
saadee198814@gmail.com

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative rod-shaped bacterium that is often responsible for causing several immune-compromised infections, burned patients, and cystic fibrosis individual hardships. *P.aeruginosa* is a bacterium with motility. In addition to flagella, *P. aeruginosa* swarming requires two exoproducts, rhamnolipids (RLs) and 3-(3 hydroxyalkanoyloxy alkanoic acids) (HAAs) to be released.[1] *P. aeruginosa* product a features apparition when grown on Blood agar medium. It shows an expanding periphery as large gray colonies and shows β-hemolysis. Colonies often have an crocodile skin apparition and exhibit a metallic bright, Rapid diagnosis of *P. aeruginosa* in culture can be make whenever the following characteristics are observed: exemplary colony morphology, Diffusible pigment production (Figure 1A), and the fruity oder of it, and oxidase positivity[2]. *Pseudomonas aeruginosa* is a main factor of nosocomial contagion and are accounted for around 90,000 deaths annually and related with urinary tract infection(UTI), linked with catheter(CAUTI)[3]. Opportunistic infection can be caused via duo methods; wound or burn contaminated or nosocomial bacteria[4]. Hospitalized patients especially those with immunocompromised infection may be displayed to contaminated devices and tools or fluids with these bacteria which cause infections[5,6]. *P. aeruginosa* is a non-fermentative aerobic that derives its energy from oxidation instead of carbohydrate fermentation. Despite the fact that more than 75 different organic compounds are able to be used. *P. aeruginosa* tolerates high salt concentrations, colorants, poor antiseptics and many antibiotics widely used. The *P. aeruginosa* lipopolysaccharide is much less toxic than that of the Gram-negative rods. The LPS of most *P. aeruginosa* strains includes , in addition to heptose, 2-keto-3-deoxyoctonic acid and hydroxy-fatty acids[7]. *P. aeruginosa* producing several components expected to promote host tissue colonization and infection. In addition to lipopolysaccharide (LPS), exotoxin A, leukocidin, extracellular slime, proteases, phospholipase and many other enzymes, these components along with a variety of virulence factors[8]. These components, together with a diversity of virulence factors, in addition to lipopolysaccharide (LPS), exotoxin A, leukocidin, extracellular slime, proteases, phospholipase, and many other enzymes (Table 1)[9]. Shape the most clinically important of *P. aeruginosa* bacteria among the NFBs. An abnormal mucoid morphotype of *P. aeruginosa* is regularly recovered from respiratory secretions of patients with cystic fibrosis (CF) who are chronic *P. aeruginosa* infections (figure 1B). The mucoid morphotype is due to the output of massive quantities of a polysaccharide (called alginate) that envelope the cell. The production of alginate is at the latest responsible for the insufficient prognosis and huge mortality rates among patients with CF[10].

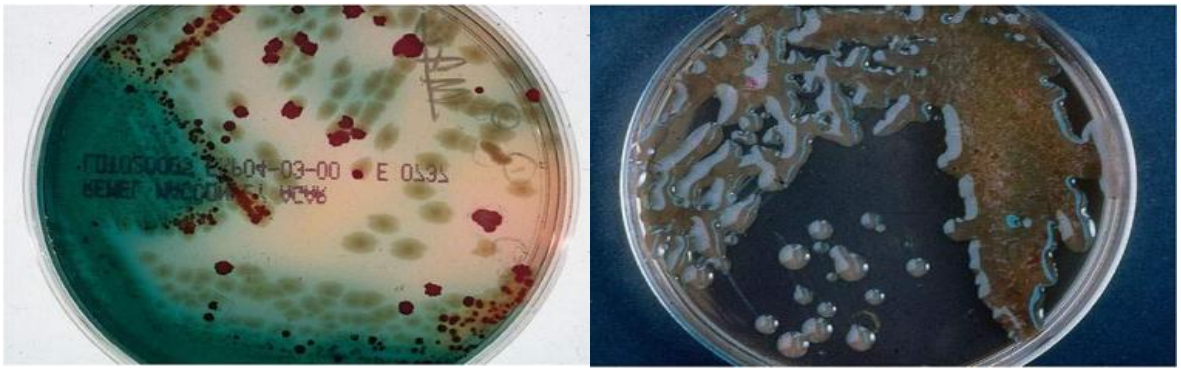

Figure 1: A-MacConkey agar plate demonstrating growth of two colony types. **B-**MacConkey agar plate demonstrating growth of a mucoid variety of *P. aeruginosa*.

Table 1: Virulence Factors of *Pseudomonas aeruginosa*

Virulence Factor	Biologic Activity
Alginate	Capsular polysaccharide that adheres to the lung epithelial cell surfaces and forms biofilms by infecting bacteria.
Pili	Surface extensions that let adherence of organismto GM-1 ganglioside receptors on host epithelial cellsurfaces
Neuraminidase	Eliminate sialic acid residues fromGM-1 ganglioside receptors, ease the bound of pili
Lipopolysaccharide	Produces endotoxin, will lead to form sepsis syndrome: fever, shock, oliguria, leukopenia or leukocytosis, spread intravascular coagulation, metabolic abnormalities
Exotoxin A	Tissue demolition, suppression of protein synthesis; intermittent cell activity and macrophage reaction
Enterotoxin	Sporadic normal gastrointestinal activity, will causing diarrhea
Exoenzyme S	Suppression protein synthesis
Phospholipase C	Destruction of cytoplasmic membrane; destroys pulmonary surfactant; inhibitions opsonins
Elastase	Split the immunoglobulins and complement ingredient, and cripple the neutrophil activity
Leukocidin	Suppress neutrophil and lymphocyte role
Pyocyanins	Inhibit other bacteria and cripple respiratory ciliary activity; lead to oxidative destruc of the tissues, particularly oxygenated tissues such as lung

Infections with *P. aeruginosa* can be difficult to handle. A relatively narrow spectrum of antimicrobial agents is effective against P. aeruginosa involving carboxypenicillins (carbenicillin, ticarcillin), ureidopenicillins (mezlocillin and piperacillin), antipseudomonal cephalosporins (ceftazidim), monobactams (aztreonam), carbapenems (imipenem and meropenem), quinolones (ciprofloxacin and levofloxacacin) and aminoglycosides (gentamicin, tobramycin, and amikacin) Almost all strains, like ampicillin, cefuroxime, and cefotaxime, are resistant to other penicillins and cephalosporins. (Table 2)[11]. *P. aeruginosa* has the potential to bear multi-resistance plasmids, which has contributed to the emergence of some *P. aeruginosa* strains resistant to all active antibiotics [12]. Bacteria can stick to medical device surface by flagella protein, type IV pili, and surface adhesion or by chemical and physical interaction and display alteration in phenotype, growth rates, metabolic activity, gene expression and product of protein[13].

Table 2: Disk diffusion quality control (QS) ranges for *P. aeruginosa* according to CLSI 2020[11].

Name of Antibiotic	Disk Content	Disk Diffusion QC Ranges, mm
		***P. aeruginosa* ATCC® 27853**
Amikacin	30 µg	18–26
Azlocillin	75 µg	24–30
Aztreonam	30 µg	23–29
Carbenicillin	100 µg	18–24
Cefepime	30 µg	25–31
Cefiderocol	30 µg	22–31
Cefoperazone	75 µg	23–29
Cefotaxime	30 µg	18–22
Ceftazidime	30 µg	22–29
Ceftizoxime	30 µg	12–17

Ceftobiprole	30 µg	24–30
Ceftriaxone	30 µg	17–23
Ciprofloxacin	5 µg	25–33
Clinafloxacin	5 µg	27–35
Colistin	10 µg	11–17
Delafloxacin[d]	5 µg	23– 29
Doripenem	10 µg	28–35
Enoxacin	10 µg	22–28
Ertapenem	10 µg	13–21
Fleroxacin	5 µg	12–20
Garenoxacin	5 µg	19–25
Gatifloxacin	5 µg	20–28
Gemifloxacin	5 µg	19–25
Gentamicin[f]	10 µg	17–23
Grepafloxacin	5 µg	20–27
Imipenem[g]	10 µg	20–28
Levofloxacin	5 µg	19–26
Levonadifloxacin	10 µg	17–23d
Lomefloxacin	10 µg	22–28
Meropenem	10 µg	27–33
Moxalactam	30 µg	17–25
Moxifloxacin	5 µg	17–25
Netilmicin	30 µg	17–23
Norfloxacin	10 µg	22–29
Ofloxacin	5 µg	17–21
Piperacillin	100 µg	25–33
Plazomicin	30 µg	15–21
Polymyxin B	300 units	14–18
Sparfloxacin	5 µg	21–29
Tebipenem[g]	10 µg	20–26
Ticarcillin	75 µg	21–27
Tigecycline	15 µg	9–13
Tobramycin	10 µg	20–26
Trovafloxacin	10 µg	21–27
Ulifloxacin (prulifloxacin)[l]	5 µg	27–33

METHODS

The detail for this review article was collected from previous Iraqi studies on *P. aeruginosa* antimicrobial activity. Several techniques have been used in this study, including identification of virulence genes using the technique of polymerase chain reaction (PCR), and use of minimum inhibitory concentration (MIC) of antibiotics for the determination of *P.aeruginosa* resistance to them.

Iraqi Studies for Virulence Factors and Antibiotics Resistance

Resistance of *P. aeruginosa* for the antibiotics

Bacteria exhibit numerous antibiotic resistance mechanisms like reduced permeability, Efflux expression. The production and targeting of antibiotic inactivating enzymes amendments. *P. aeruginosa* has most of these known exhibits Resistance mechanisms chromosomally from both intrinsic resistance determinants encoded or imported genetically Influencing the large antibiotic groups (Table 3)[14].

Table 3: Mechanisms of *P. aeruginosa* Chromosomally encoded or imported resistance.

Location	Resistance mechanisms	Targeted antibiotics	Type of resistance
chromosomal	AmpC–type cephalosporinase	β-lactams	Antibiotic inactivation
	Class D oxacillinase OXA-50	β-lactams	Antibiotic inactivation
	Aminoglycosides inactivating enzymes	Aminoglycosides	Antibiotic inactivation

	Efflux systems (overexpression)	Multiple antibiotic classes	Efflux systems
	Decreased membrane permeability	Multiple antibiotic classes	Membrane impermeability and purines
	DNA gyrase and topoisomerase IV	Fluoroquinolones	Target modification
	LPS modification	Colistin	Target modification
Imported	Class A serine β-lactamases (PSE, CARB, TEM)	β-lactams	Antibiotic inactivation
	Class A serine ESBL (TEM, SHV, CTX-M, PER, VEB, GES, IBC)	β-lactams	Antibiotic inactivation
	Class D ESBL (OXA-types)	β-lactams	Antibiotic inactivation
	Class B Metallo-β-lactamase (IMP, VIM, SPM, GIM)	Carbapenems	Antibiotic inactivation
	Class A serine carbapenemase (KPC)	Carbapenems	Antibiotic inactivation
	Class D carbapenemase (OXA-types: OXA-40)	Carbapenems	Antibiotic inactivation
	Aminoglycosides inactivating enzymes	Aminoglycosides	Antibiotic inactivation
	Ribosomal methyltransferase enzymes	Aminoglycosides	Target modification

In a study, the sensitivity of *P. aeruginosa* to five antibiotics was tested: ceftazidime (CAZ), azithromycin (AZM), amoxicillin-clavulanate (AMC), ciprofloxacin (CIPR), and cefoxitin (CTX) using the disc diffusion method, where the isolates showed resistance of 66.6. % For (CAZ) and (CIPR), (CTX), 100% for (AMC), and 33.3% for (AZM)[15]. One hundred and eighty-one insulates of *P. aeruginosa* were gathered from the ear, wounds, and urine. After the diagnosis, the highest percentage of ear swabs appeared compared with the samples of wounds and urine, after which the sensitivity of these isolates was tested using different antibiotics, where the highest rate of resistance to antibodies ampicillin (100%), followed by Cephalothin (92.82%), Cefotaxem (84.53%), Gentamycin (69.61%) and Trimethoprim (67.99%), Carbencillin (67.95%), Ceftriaxon (24.86%), Ciprofloxacin (20.99%), Cefamandol (19.33%), Tobramycin(14.91%), Amikacin(14.3%), and Piperacillin (12.7%). While each of the following antibiotics, Piperacillin, Amikacin, and Tobramycin, showed a good effect against *P. aeruginosa*, then the ability of these bacteria to produce β-lactamase was revealed by a diffusion method double disk, where 13 of the 50 isolates produced this enzyme[16].

In another study to neutralize some antibiotic resistance genes of *P. aeruginosa* by Sodium Dodecyl Sulphate and Ethidium bromide, 22 urine isolates were collected from Diwaniyah Hospital and these isolates were diagnosed using a VITEK 2 system. After the diagnosis, sensitivity to a number of antibiotics was examined, where isolates demonstrated resistance to nalidixic acid, oxacillin, vancomycin, ampicillin and ciprofloxacin but instead kanamycin and tetracycline sensitivity. No neutralization of 700 µg / ml Ethidium bromide was observed for all isolates, while the concentrates dissolved when using sodium dodecyl sulfate at a concentration of 1, 0,9, 0,8% W / V. The isolates were susceptible to (0.5,0.6 and 0.7) percent concentration of sodium dodecyle sulphate (SDS) were 10 mg of nalidixic acid (35.1, 94.5 and 100) percent, 30 mg of vancomycin (59.4, 86.4 and 100) percent, 30 mg of kanamycin (97.2, 100,100) percent and 30 mg of tetracycline (75.6, 100 and 100) percent respectively, no Oxacillin 30 mg, Ciprofloxacin 5 mg and Ampicillin 10 mg sensitivity [17]. The researcher[18] collected twenty-five isolates belonging to *P. aeruginosa* from: wounds, burns, inflammation of the respiratory tracts,and UTI, and tested the sensitivity of these isolates to 13 antibiotics. The findings suggested that the majority of bacterial isolates were immune to most antibiotics (Amoxicillin (25 µg); and (30 µg) for the following antibiotics; Ak: Amikacin CTX: Cefotaxime; CRO: Ceftriaxone; CAZ: Ceftazidime; TE: Tetracycline ; NA: Nalidixic acid and KF: Cephalothin; and 10 µg for each antibiotics IMP: Imipenem; LEV: Levofloxacin; and MEM: Meropenem; finally the (5 µg) of the CIP: Ciprofloxacin and CN: Gentamicin antibiotic. *P. aeruginosa* is considered one of the main factors of blood poisoning, [19]collected 200 samples from burn patients who suffer from blood poisoning, where 34 isolates of the *P. aeruginosa* emerged, which are prevalent among other races, and the study also showed that burn poisoning usually occurs during The first week, especially on the seventh day.

Detection of virulence factor genes for *P. aeruginosa*

The genes encoding the virulence factors of *Pseudomonas aeruginosa* have been studied which have a significant role in the antibiotic resistance of these bacteria (Table 4)[20].

Table 4: Types of genes in Pseudomonas aeruginosa and their function[20].

Name of gene	Function	Name of gene	Function
lasB	Encoding the elastase	***gyrB***	Encodes the subunit B protein of DNA gyrase
ETA	Exotoxin A	***Exo S***	ADP-ribosyltransferase
qacEΔ1	Resistance for biocide	***acc (3 ') - I***	aminoglycosideN-acetyltransferase
***rhlA, rhlR, rhlI, rhlAB, lasR* and**	Encoding the rhamnosyltransferase	***aad A1***	streptomycin adenyltransferase
nan1	Sialidase	***aph (3') - IIb***	aminoglycoside3-phosphotransferase IIb
opr I and opr L	Natural antibiotic and antiseptic resistance	***algD* and *lasI***	Catalytic activity
blaCARB	Resistance to Carbenicillin	***arr-2***	Rifampin resistance
plcH	Encoded to phospholipase	***aprA***	alkaline protease
MexABOprM, MexXY-OprM, MexEFOprN* and *MexCD-OprJ	Resistance to gentamicin	***phzA1***	Antibiotic biosynthetic process
blaVIM, blaIMP, blaNDM,	Carbapenem-resistant genes	***lecA***	D-galactose specific lectin

blaSIM,* and *blaSPM			
exoU	Proton acceptor and Nucleophile	***toxR***	Positive regulation of toxA gene transcription
fliC	Flagellin		

The *lasB* gene was detected in 75 *P. aeruginosa* isolated from burn infections, wound, otitis, blood, and urinary tract infections. The gene was detected for all isolates, where it was found that 23 isolates out of 28 isolates of ear infections, 19 of 23 isolates for burn infections, all wound isolates, 5 urinary tract infection isolates from 8 isolates, and all six blood isolates were carrying the *lasB* gene, which has an important role in encoding the enzyme elastase, which has high efficiency for virulence of bacteria in the necrosis process[21]. In another study of *P. aeruginosa* in which the virulence *ETA* and *gyrB* genes were detected, twenty-four isolates of these bacteria were collected from a total of 70 samples of the wounds and burn infections, and the DNA of these isolates was extracted after which the two genes were detected, where the results showed that all isolates carry the *gyrB* gene. As for the *ETA* gene, it was present in 74% of the isolates, where this method was used for the rapid detection of *P. aeruginosa* because the *gyrB* gene was not present in other bacterial samples[22].

A study conducted by[23] showed the virulence of *P. aeruginosa* for 35 isolates from Azadi Teaching Hospital in Kirkuk city where a number of virulence factors, were estimated β-lactam enzyme, protease, lipase, lecithinase, deoxyribonuclease, gelatin liquefaction, haemolysine, congo-red binding, urease, capsule presence. where the results showed that all isolates were produced the β-lactamase, but they differed in their production of other factors. Fifty-two isolates were collected from Najaf Hospital for *P. aeruginosa*, where the sensitivity of these isolates was examined for 16 antibiotics, and the results showed that 33 isolates had multiple resistance, by 63.5% divided into two parts, 28.8% of isolates (MDR) and 34.6% extensive drug resistance (XDR), it was found. The increase in resistance to carbapenem antibiotics confirms that there are a difficulty and problem in treating infections with these bacteria[24]. In a study conducted by[25], the *qacEΔ1* gene was detected for *P. aeruginosa*, where 69 isolates were collected from clinical cases that included wounds and burns, and the band size was 285 bp. Analysis of the genetic tree that the isolates had the same genetic dimension. In a study conducted by the researcher[26] twelve environmental samples of *P. aeruginosa* were isolated from water river and also seven Clinical isolates from various sources included burn infections, ear infections, and respiratory tract infections, where 13 antibiotics were used and a minimum inhibitory concentration (MIC) test for all environmental and clinical isolates and biofilm composition was evaluated for these isolates. AgNps was prepared using the Myrtus communis leaf method, and then the MIC of AgNps was measured by a tube method. The effect of 0.5 x MIC of AgNps on biofilm formation was checked using the microtiter plate method. Also, genes (*rhlA, rhlR, rhlI, lasR, lasI, lasB, phzA1*) were used the Polymearse chain reaction and gene expression of two genes *lasI* and *rhlR* was also used the Real-time PCR for detected. The results appeared that the resistance of samples isolate from patients to antibiotics was higher than that of environmental isolates, and the lowest inhibitory concentration of AgNps against the first environmental isolate was 150 g / ml while it was 450 g / ml against other environmental isolates, also the MIC of clinical isolates was 150 g / ml for first clinical isolates while For other isolates, 450 µg / ml. The study also showed that all isolates have the ability to produce biofilm, as clinical isolates showed their ability to produce biofilm (0.299 ± 0.08) higher than environmental isolates (0.245 ± 0.05). The study also showed that all environmental and clinical isolates were carriers of the seven genes, which are related to Quorum sensing. When assessing gene expression by Real-time PCR of the genes *lasI* and *rhlR*, results showed that exposure to a lower dose than a dose of AgNps inhibition significantly decreased gene expression in the clinical and environmental isolates of these bacteria, as it was observed that there was a significant decrease in the *lasI* gene in clinical samples and environmental after exposure to 0.5 × the MIC of silver nanoparticles .

The effect of Nd: Yag Laser was also tested on some virulence factors of *P. aeruginosa*. Clinical samples were collected from wound infections, burns, ear infections, and environmental isolates. All isolates were exposed to the laser, but before the exposure the DNA was extracted and also after the exposure process, the results appeared a obvious impact of the laser On the virulence genes (*Tox A, Opr L* and *Exo S*) due to their loss of binding sites compared to control isolates, as it was found that the laser has an effect on these bacteria, and we can use this method in the sterilization and treatment process[27]. Virulence genes were detected (*opr I, opr L*) of 40 *P. aeruginosa* isolates from different sources (burns, wounds, ear, and urinary infections). The results showed that all isolates were carriers of the mentioned virulence genes and are also very important for the rapid detection of *P. aeruginosa* infections[28].

Also, in another study, 100 swabs with patients suffering from burns and wounds infections were taken from Al-Diwaniyah hospital. The results showed that 27 isolates were affiliated with *P. aeruginosa* after which the sensitivity of the aminoglycoside group was tested using the diffusion method and measuring the diameter of inhibition. It was found that there was a variation in the results, as the bacteria showed high resistance to some antibiotics and less resistance to others, and based on these results, 13 isolates appeared that had MDR. Three genes (*acc (3 ') - I, aph (3') - IIb, and aad A1*) were detected via the PCR, the results appeared that most of the *P. aeruginosa* were carriers of the gene *acc (3 ') - I* (46%). In Individually, about 23% or mixed, 15% *acc (3 ') - I + Aph (3') Iib and acc (3 ') - I + aadAI* by 7.7%, while one isolate was carrying the *aadAI* gene alone[29]. In another study, the gene (*arr-2, acc (3')- I and* bla_{CARB}) was detected in 48 isolates from Hilla Hospital for *P. aeruginosa*, after the antibiotic susceptibility test was done showed high resistance to most of the antibiotics while they were susceptible to both IPM and MRP. The results of the detection of genes showed that the highest rate of the gene *arr-2*, followed by the *aac (3) I* gene, and the lowest percentage of the bla_{CARB} gene[30]. Most of the genes responsible for virulence factors in the *P. aeruginosa* were studied by[31] for 286 isolates of these bacteria collected from different sources, including the virulence genes (*plcH, algD, rhlI, exoS, exoU, lasR, toxA, aprA, rhlAB, fliC, lecA, toxR, lasI, opri, oprL, rhlR, nan1, lasB*). The results showed the prevalence of virulence genes for most *P. aeruginosa* isolates: *lasI* 3.5%, *lasR* 2.0%, *rhlI*, 2.4%, *rhlR* 4.3%, *toxA* 9.9%, *aprA* 2.1%, *rhlAB* 2.6%,

plcH 10.5%, *lasB* 10.6%, *fliC* 2.5%, *lecA* 4.7%, *algR* 10.4%, *toxR* 4.7%, *oprI* 6.4%, *oprL* 7.5%, *nan1* 2.0%, *exoS* 9.4%, *exoU* 4.5%. The highest rate of blood infections was followed by burn ,Urinary tract infection, and finally wound injfections.

[32]Investigated the presence of the Efflux MexX gene in 54 isolates of *P. aeruginosa* from various sources, including samples of wounds, burns, urinary tract infection, otitis, sputum, and respiratory tract infection, then molecular detection was done using housekeeping gene (*rpsL*). The results showed 48 out of 54 isolates were carrying the efflux system MexX gene between resistance and intermediate, while 6 samples showed sensitivity due to not having the gene. As well, the spread of *P. aeruginosa* resistant to carbapenem antibiotics in hospitals is an important and dangerous case, as [33] isolated 50 samples from the Specialized Surgery Hospital in Erbil from the intensive care unit and patients with advanced lung infections and associated with the ventilator, samples were diagnosed by amplifying the *oprL* gene. A VITEK 2 system was also used to test for sensitivity to antibiotics. Carbapenemase-producing *P. aeruginosa* samples were then tested for the existence of metallo β-lactamase encoding the genes bla_{VIM}, bla_{IMP}, *and* bla_{NDM}. The results showed 76% [MDR] phenotype consists of isolates resistant to more than one antimicrobial agent in three or more groups of antimicrobials, 20% extensively drug-resistant [XDR] phenotype involves isolates resistant to more than one antibacterial agents in all classes of antimicrobials, except for two or less, and 4% pan drug resistance [PDR] Phenotype contains isolates resistant to every antibacterial agents in all classes of antimicrobials, 12 isolates also showed positive for CRPA, and the bla_{VIM} gene appeared more widespread, followed by the bla_{NDM} gene and the least bla_{IMP} gene. As for the [34], studied the spread of metallo-β-lactamase produced by *P. aeruginosa* in diabetic foot infections in Najaf, where 97 isolates of these bacteria were collected, where the sensitivity test for 13 antibiotics was tested by the method of spread and the phenotype was confirmed. By the tablet test also the double-disc synergy test, the Hodge test, and finally the CHROM agar, the genes with the phenotype producing MBL were also detected. These genes are as follows: bla_{IMP}, bla_{NDM}, bla_{SIM}, bla_{SPM}, *and* bla_{VIM} by PCR technique. Twelve isolates showed that were producing MBL by Hodge test and ten samples with CHROM agar KPC agar, while 9 isolates yielded carbapenemase, characterized by the two-disc synergy test of imipenem and ceftizoxime. The results of the detection of genes from among the 12 phenotypes that were producing MBL showed that 4 isolates gave a positive result for the bla_{VIM} gene, while three isolates were for the bla_{IMP} gene and were not containing other genes.

Detect of Pyocyanin dye and Biofilm

In another study to determine the mastery of *P. aeruginosa* for product pyocyanin dye for 50 isolates from Kirkuk General Hospital, the results showed that 43 isolates produced this dye on Muller-Hunton agar medium, as well as the nutrient agar, while 30 isolates produced the dye on MacConkey agar medium and 24 isolates on the medium of blood agar (Figure 2) [35].

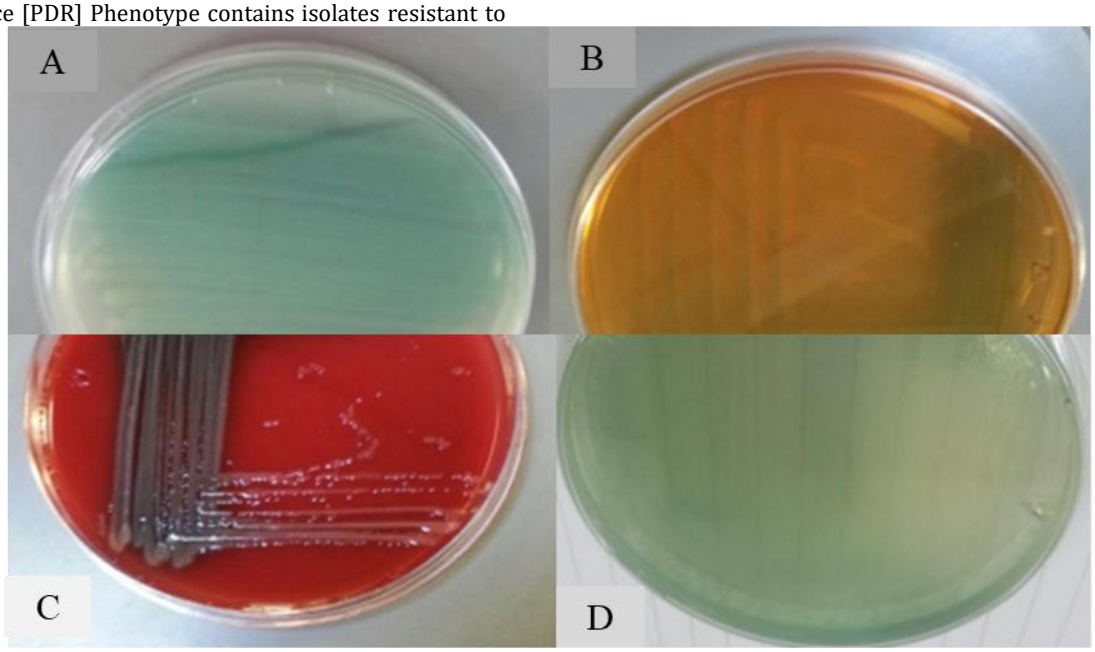

Figure 2: Pyocyanin dye products from *P.aeruginosa* on: A- Nutrient agar B- MacConkey agar C- Muller-Hunton agar D- Blood agar.

P. aeruginosa were diagnosed with a number of clinical samples using the *16srDNA* gene, where it was found that all 75 isolates were carrying this gene and the band size was 956 bp. Also, the sensitivity test was examed for a number of antibiotics, where the isolates showed resistance to Kanamycin, Ceftazidime and less to Gentamicin, Tobramycin, Piperacillin, Ofloxacin, Ciprofloxacin, Norfloxacin and showed the least resistance to Aztreonam and Imipenem, respectively. The results showed that all isolates were producing Hemolysin while 61 isolates had Protease. As for the production of Pyocyanin, 78.66% of the isolates produced this dye, as well as for Amylase and finally, 54 isolates showed their ability to produce biofilm[36]. The ELISA technique and the linking of crystal violets, which relied on the optical density of violets associated with the biofilm, was used to investigate the ability of different bacterial species to produce it, as 10 samples of *P. aeruginosa* were collected from burn and wound infections at a wavelength of 620 nanometers, the results appeared that there was a diversity in the bacterial isolates. The most productive biofilms were isolated from the diabetic ulcers, as the study proved that there is a plasmid and chromosomal role in regulating the biofilms formation[37].

Antibacterial activity of some plant extract and aromatic oil

In a study to find out the effect of fenugreek oil extract on *P. aeruginosa*,[38] Twenty-eight isolates of these bacteria have been obtained from skin diseased patients from Al-Yarmouk Teaching Hospital, after which sensitivity was examined using 14 antibiotics via the VITEK 2 system. *P. aeruginosa* has been found to be extremely Trimethoprim / Sulfamethoxazole resistant while they were sensitive to Amikacin. As for the effectiveness of the fenugreek extract, the minimum inhibitory concentration was measured using the liquid micro-dilution ways and mice were used in the form of five groups (control, induction, treatment with the extract alone, using Gentamicin alone, and a combination treatment between the extract and Gentamicin, then the tissues get examed after the treatment seven days. The results showed that there are suppressive efficacy of the fenugreek extract alone, as well as when we mix it with gentamicin compared to when using the antibiotic alone (Table 5). Other study by [39] prepared pomegranate extract rich in ellagic acid, where the method included the extraction with methanol containing 20% water V/V under thermal sublimation conditions in order for the acid to be purified by TLC, then the compound was determined using thin layer chromatography (TLC), high-perference liquid chromatography (HPLC) and UV and the functional groups of the acid were determined by means of the FTIR spectro technique determined the inhibitory activity of this extract from the dried pomegranate fruit powder by means of a petri dish for some bacteria. It was effective against *Staphylococcus aureus* and *E. coli* and *P. aeruginosa* were not affected by the extract and were resistant to it.

Table 5: Impact of fenugreek essential oil on multidrug resistance isolates *P. aeruginosa* alone and in combination with gentamycin.

Isolates no.	Fenugreek MICgm/100µl	Fenugreek 1/4 MIC	Gentamycin 1/4 MIC	1/4+1/4 MIC	1/2+1/2 MIC	FIC Values FIC/Interpretation	
1	1.2	+	+	+	-	1	Indifference
2	1.2	-	+	-	-	0.5	synergism
3	0.6	+	-	+	-	1	Indifference
4	1.2	-	+	-	-	0.5	synergism
5	0.6	+	+	-	-	0.5	synergism
6	0.6	+	+	-	-	0.5	synergism
7	1.2	-	+	-	-	0.5	synergism
8	1.2	+	-	-	-	0.5	synergism
9	1.2	+	+	-	-	0.5	synergism
10	0.6	+	+	+	-	1	Indifference
240 mg 0.77		240 mg 0.77	0.00325 mg	2.4 mg 0.77			

– (no growth), + (growth), fractional inhibitory concentration (FIC) was determine as follow: ≤0.5 = synergism, 0. 5-< 1= additive, 1- <4 = indifference, ≥4 = antagonism, P-value less than 0.05 were considered significant.

In anthor study on the effectiveness of red cabbage extract on the uricase produced by *P. aeruginosa* carried out by [40] where this enzyme was separated and the highest activity of this enzyme was measured at 35 ° C and a pH of 8.5, where the results showed that the Red cabbage extract has had the effect of inhibiting the enzyme uricase and may be used in the future in the treatment of gout.

One hundred and twenty-five *P. aeruginosa* isolates were isolated from Al-Hilla Teaching Hospital. The sensitivity test for these isolates was tested, as the agents showed resistance to these antibiotics except for the colistin, Ampicillin (80%) Carbenicillin (98%) Chloramphenicol (99%) Rifampin (97%), Imipenem (35%) meropenem (36%), Tetracycline (89%) and colistin (0%). In this study, essential oils were used, including Syzygium aromaticcum (clove oil), Allium sativum (Garlic oil), Origanum majorana (majora oil), and Cinnamonumn zeylanicum (cinnamon oil). The results showed that cinnamon oil was a strong inhibitor of isolates that possessed multiple resistance compared to other oils[41].

CONCLUSION

We conclude from this review that *P. aeruginosa* possesses wide resistance to antibiotics, and most studies have indicated the use of alternatives to antibiotics such as aromatic oils and plant extracts. Also, Most studies also showed that there is development in virulence factors and their ability to break down antibiotics.

Acknowledgment

Thanks for Al-Maarif university college for its continuous support to researchers, we also thank the researchers whom we mentioned.

REFERENCES

1. Tremblay, J., Richardson ,A.P., Lépine, F. and Déziel, É. Self-produced extracellular stimuli modulate the Pseudomonas aeruginosa swarming motility behavior. Environ Microbiol. (2007).9 (10): 2622-2630.
2. Clinical and Laboratory Standards Institute. Abbreviated Identification of Bacteria and Yeast. 2nd Ed. CLSI Approved standard M35-A2. Wayne, PA: Clinical and Laboratory Standards Institute. (2008).
3. Jacobsen, S. á., Stickler, D., Mobley, H. and Shirtliff, M. Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis. Clin Microbiol Rev. (2008). 21(1): 26-59.
4. Dutkiewicz, J., Mackiewicz, B., Lemieszek, M. K., Golec, M., & Milanowski, J. Pantoea agglomerans: a mysterious bacterium of evil and good. Part III. Deleterious effects: infections of humans, animals and plants. Ann Agric Environ Med. (2016). 23(2) : 197–205.
5. Shubov, A., Jagannathan, P. and Chin-Hong, P. Pantoea agglomerans pneumonia in a heart–lung transplant recipient: case report and a review of an emerging pathogen in immunocompromised hosts. Transpl Infect Dis. (2011). 13(5): 536-539.
6. Boszczowski, I., de Almeida Júnior, J. N., de Miranda, E. P., Freire, M. P. et al. Nosocomial outbreak of Pantoea agglomerans bacteraemia associated with contaminated anticoagulant citrate dextrose solution:

new name, old bug. J Hosp Infect. (2012). 80(3): 255-258.
7. Baron,S. Medical Microbiology. 4th edition. University of Texas Medical Branch at Galveston. (1996).
8. Bodey GP, Bolivar R, Fainstein V, et al. Infections caused by Pseudomonas aeruginosa. Rev Infect Dis. (1983). 5:279–313.
9. Moss RB Cystic fibrosis: pathogenesis, pulmonary infection, and treatment. Clin Infect Dis. (1995). 21:839–851.
10. Stoltz DA, Meyerholz DK, Welsh MJ. Origins of cystic fibrosis lung disease. N Engl J Med (2015).372(4):351–362.
11. CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute. (2018). Update M100, 30th ed , 2020.
12. Livermore DM Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: our worst nightmare? Clin Infect Dis. (2002). 34:634–640.
13. Lister, J. L. and Horswill, A. R. Staphylococcus aureus biofilms: recent developments in biofilm dispersal. Front Cell Infect Microbiol. (2014). 4: 178.
14. Bassetti M, Vena A, Croxatto A, Righi E, Guery B. How to manage Pseudomonas aeruginosa infections. Drugs in Context (2018). 7: 212527. DOI: 10.7573/dic.212527.
15. Mohammed Hayder Hashim and Marwa Hameed AlKhafaji. Antibiotics resistance among clinical isolates of Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli. Al-Nisour Journal for Medical Sciences. (2019). 1, (1). 65-71.
16. Adnan kreem Al-salamy and Emad Sadiq Ali Al-Hilli. Antibiotics Susceptibility pattern of Pseudomonas aeruginosa that isolated from ear, wound and urine samples. journal of the college of basic eduction. (2012). (76).101-110.
17. Zeena Fouad Saleh. Curing of some Antibiotic Resistance by the Action of Sodium Dodecyl Sulphate and Ethidium bromide in Pseudomonas aeruginosa Isolated from urine of human, cow meat and horses wound. Kufa Journal For Veterinary Medical Sciences. (2017). (8) 2. 115-123.
18. Yahia A. Abbas, Bassam Y. Khudaier and Hind A. Salih. Detection of Multidrug Resistant Pseudomonas spp. in Clinical Cases and Hospital Environments at Thi-Qar Province. Journal of College of Education for Pure Sciences. (2014). 4. 205-213.
19. Ghassan A. Hassan. Sepsis in Burn Patients. Raf. J. Sci. (2019). 28, 4. 13-21.
20. https://www.uniprot.org/uniprot/O34208.
21. Rana M. Abdullah Al-Shwaikh and Abbas Falih Al-Arnawtee. Detection of lasB Gene of Pseudomonasaeruginosa Causing Different Infection. 1st Scientific International Conference, College of Science, Al-Nahrain University. (2017). Part II,48-52.
22. Khulod Ibraheem Hassan and Saman Rafeeq Abdullah. Detection of Pseudomonas aeruginosa in Clinical Samples Using PCR Targeting ETA and gyrB Genes. Baghdad Science Journal. (2018). 15(4). 401-405.
23. Siham Sh. AL-Salihi and Abbas Y. Hasan. Detection of some virulence factors in Pseudomonas aeruginosa associated with diarrhea in Kirkuk City. Kirkuk University Journal. (2015). (10) 1. 78-89.
24. Alaa Abdulhussein Rasool and Ali M. Almohana. Dissemination Multi-Drug and Carbapenems Resistant Pseudomonas aeruginosa among Clinical Isolates in Najaf Hospitals. Al-Kufa University Journal for Biology.(2017). (9)3. 146-155.
25. Shahla Najim Abed Al-Azzawi and Rana Mujahid Abdullah. DNA Sequences of qacEΔ1 Gene in Pseudomonas Aeruginosa Isolated from Wounds and Burns Infections. Kirkuk University Journal. (2019). 14(3). 10-20.
26. Huda Abbas Mohammed and Ayaid Khadem Zgair. Effect of silver nanoparticles on biofilm and gene expression of quorum sensing genes in Pseudomonas aeruginosa. College of Science at University of Baghdad. (2020).
27. Aidah Abd Al-doori , Awatef Saber Jasem and Adnan F. AL-Azzawie. Effects of Nd:Yag Laser on some virulence factor genes of Pseudomonas aeruginosa bacteria. Tikrit Journal of Pure Science. (2020). 25 (2).86-92.
28. Rana Kadhim Mohammed and Ruaa Alwan Ahmad Molecular. Detection of Virulence Factors Genes in Pseudomonas aeruginosa isolated clinically from Different Infections Source. Current Research in Microbiology and Biotechnology. (2017). 5, 5.1278-1284.
29. Majida M. Meteab Alshammari, Miaad K. Alkhudhairy and Ahlam Ali Soghi. Prevalence of aminoglycoside modifying enzyme genes in Pseudomonas aeruginosa isolated from Burn Centers in Iraq. Drug Invention Today. (2019). 11 12. 3149-3154.
30. Amal Talib Atiyah Al-Sa'ady And Naher H. S. Molecular Detection Of Multi-Drug Resistant P. Aeruginosa Isolated From Neonatal Infections. International Journal of Medicine and Pharmaceutical Sciences. (2014). 4, 5.69-76.
31. Lena Fadhil, Ali Hussein Al-Marzoqi2, Zahraa Mohammad Al Taee and Ammar A Shalan. Molecular and Phenotypic Study of Virulence Genes in a Pathogenic Strain of Pseudomonas aeruginosa isolated from various clinical origins by PCR: Profiles of genes and Toxins. Research Journal of Pharmaceutical, Biological and Chemical Sciences. (2016). 7(1).590-598.
32. Suhad H. Friyah and Marrib N. Rasheed. Molecular Study of Efflux MexX Gene in Pseudomonas aeruginosa Isolated from Iraqi Patients. Iraqi Journal of Biotechnology. (2018). 17, 3. 92-99.
33. Soza Tharwat Baban. Molecular Detection of Carbapenemase- Producing Pseudomonas aeruginosa Isolated from Intensive Care Units of Surgical Specialty Hospital in Erbil City. Medical Journal of Babylon. (2020). 17,2. 185-193.
34. M. K. Alkhudhairy and M. M. M. Al-Shammar. Prevalence of metallo-β-lactamase–producing Pseudomonas aeruginosa isolated from diabetic foot infections in Iraq. New Microbes and New Infections. (2020). 35 Number C. 1-6.
35. Tara Fakhraddin raheem- Al-Jaff , Salah. S. Zain al-abdeen and Najdat .B. Mahdi. Isolate the bacteria Pseudomonas aeruginosa from different clinical samples and study the effect of pyocyanin pigments of some pathogenic bacterial. Kirkuk University Journal. (2016). (11) 4. 215-226.
36. Rana M. Abdullah and Abbas F. Mehdi. Identification of Pseudomonas aeruginosa From Clinical Specimen by Using 16S rDNA Gene. Biotechnology research center journal(2016). (10)1. 45-49.
37. Omar Nama Flaih, Laith Musleh Najeb and Rana Kadhim Mohammad. Determine the Biofilm Formed

by Using ELISA Technology for Gram-Negative Bacteria Isolated from Wounds and Burns Infections, and the Study of the Production of the Biofilm Molecularly. Ibn Al-Haitham J. for Pure & Appl. Sci. (2017). 30 (1). 325-338.
38. Maysaa A. Abdul Kahaleq, Ahmed R. Abu-Raghif and Shurooq R. Kadhim. Antibacterial activity of Fenugreek essential Oil against Pseudomonas aeroginosa: In vitro and in vivo Studies. Al-Nahrain College of Medicine. (2015). 13(3). 227-234.
39. Hiba H. R. , Eesa. M. Thalij and Sundus J. M. Demodulation and purification of ellagic acid from pomegranate fruit and study antimicrobial activity against the pathogenic bacteria. Tikrit Journal of Pure Science. (2019). 24 (6).51-56.
40. Mohannad S.AL-Fayyadh. Effect of aqueous red cabbage extract on uricase activity isolated from Pseudomonas aeruginosa. Iraqi Journal of Science. (2015). 56.3B.2275-2281.
41. Lubna Abdulazeem , Hadeel Alaa Alrubaei , Farah Tareq Al-Alaq , Yasir H. Al-Mawlah , Ali Shakir Obed. The Effect of Essential oils on Multi-drugs Resistant Pseudomonas aeruginosa (MDRPA). Annals of Tropical Medicine and Public Health. (2019).22(I).