Iraqi Journal of Science, 2025, Vol. 66, No. 8, pp: 3186- 3199
DOI: 10.24996/ijs.2025.66.8.12

ISSN: 0067-2904

The Mutagenic Effect of UVC Irradiation on Viability and Antibiotic Susceptibility in *Pseudomonas aeruginosa* Isolated from Clinical Cases

Bushra H Saleh, Dhafar N. Al-Ugaili*
Department of Molecular and Medical Biotechnology, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq

Received: 12/12/2023 Accepted: 22/8/2024 Published: 30/8/2025

Abstract

Pseudomonas aeruginosa is a notorious opportunistic pathogen that frequently causes hospital-acquired infections, particularly in burn wounds and urinary tract infections, and is consistently ranked among the top three most common Gram-negative pathogens associated with these types of infections. During a period of 4 months, from 8/2021 to 11/2021, 65 samples were collected from patients suffered from burns, wounds, and UTIs infections in three hospitals in Baghdad. The conventional cultural identity of the isolates was confirmed by the results of the VITEK 2 system. Molecular identification of *P. aeruginosa* isolates based on 16S rRNA gene amplification and sequencing was done, followed by testing antibiotic susceptibility profile for the isolates. Ultraviolet C (UVC) was employed to cause mutations in five *P. aeruginosa* isolates at different times (30, 90, 180, and 360 seconds) by using the UV transilluminator. The results of the mutation revealed that the viability of all five isolates decreased over time after exposure to UV light at various time periods. Notably, the P1 isolate exhibited a significantly stronger response to UVC radiation compared to the other four isolates, suggesting a unique sensitivity to this type of mutagenic stress. Mutated *P. aeruginosa* P1 isolate revealed that while bacterial cell viability decreased within time, the isolate became more sensitive toward the antibiotics ciprofloxacin, colistin, gentamycin, and Imipenem after mutation for different times. Additionally, P1 isolates showed changes in responses from resistant to susceptible toward the antibiotics Aztreonam, Cefotaxime , and Levofloxacin, while other antibiotics like Piperacillin-tazobactum ,Ceftriaxone and Ceftazidime showed the same results in P1 isolates before and after mutation.

Keywords: UVC, *Pseudomonas aeruginosa,* Burn wound, UTI, Mutation

التأثير المطفر للأشعة فوق البنفسجية على حيوية والحساسية للمضادات الحيوية لبكتيريا الزائفة الزنجارية المعزولة من الحالات السريرية

بشرى هندي صالح ، ظفر نجم عبد الامير
قسم التقنيات الحيوية الجزيئية و الطبية, كلية التقنيات الاحيائية, جامعة النهرين, بغداد, العراق

* Email: dhafar.alugaili@nahrainuniv.edu.iq

الخلاصة

تعد الزائفة الزنجارية (*Pseudomonas aeruginosa*) أحد مسببات الأمراض الانتهازية السيئة و التي تسبب في كثير من الاحيان العدوى المكتسبة من المستشفيات، ، وخاصة في جروح الحروق والتهابات المسالك البولية، وتصنف باستمرار من بين أكثر ثلاثة مسببات أمراض سلبية الجرام شيوعًا المرتبطة بهذه الأنواع من العدوى. خلال مدة 4 اشهر من اب 2021 الى تشرين الثاني 2021 تم جمع 65 عينة بكتيرية من المرضى المصابين بالحروق والجروح والتهابات المسالك البولية في ثلاثة مستشفيات في بغداد. تم التأكد من الزرع البكتيري بالطريقة التقليدية للعزلات من خلال نتائج نظام VITEK 2. تم إجراء الكشف الجزيئي لعزلات *P. aeruginosa* على أساس تضخيم وتسلسل جينات الرنا الرايبوسي (S16)، يليه الكشف عن حساسية العزلات عن طريق اختبار الحساسية للمضادات الحيوية. تم استخدام الأشعة فوق البنفسجية نوع س (UVC) لإحداث طفرات في خمس عزلات من *P. aeruginosa* في أوقات مختلفة (30، 90، 180 و 360 ثانية) باستخدام مولد الأشعة فوق البنفسجية. أظهرت نتائج الطفرة أن حيوية العزلات الخمس انخفضت مع مرور الوقت بعد التعرض للأشعة فوق البنفسجية في أوقات مختلفة. ومن الجدير بالذكر أن عزل P1 أظهر استجابة أقوى بشكل ملحوظ لأشعة UVC مقارنة بالعزلات الأربع الأخرى، مما يشير إلى حساسية فريدة لهذا النوع من الإجهاد المسبب للطفرات.. كشفت عزلة P1 *P. aeruginosa* المتحورة أنه بينما انخفضت حيوية الخلايا البكتيرية مع مرور الوقت، أصبحت العزلة أكثر حساسية تجاه المضادات الحيوية سيبروفلوكساسين وكوليستين وجنتاميسين وإيميبينيم بعد حدوث طفرة لأوقات مختلفة. بالإضافة إلى ذلك، أظهرت العزلات P1 تغيرات في الاستجابات من المقاومة إلى الحساسة تجاه المضادات الحيوية ازتريونام و سيفوتاكسيم و ليفوفلاكساسين، في حين أظهرت المضادات الحيوية الأخرى مثل بايبراسيلين–تازوباكتم و سيفرياكسون و سيفتازيديم نفس النتائج في العزلات P1 قبل وبعد الطفرة.

Introduction

Pseudomonas aeruginosa is a Gram-negative rod-shaped bacterium that belonging to the bacterial family *Pseudomonadaceae* [1]. It is commonly found in wet settings, such as sewage, water and soil. It is also present in fresh water sources, typically in tanks that have been contaminated by human or animal waste. Within hospital settings, *P. aeruginosa* has been isolated from equipment that contains or uses water. The transmission of bacteria can occur through two main routes in hospital settings: via the hands of healthcare workers or through patients directly contacting contaminated surfaces or objects [2]. *P. aeruginosa* pathogenicity occurs due to the ability of bacteria to produce a wide range of virulence factors. These characteristics enabled the bacterium to achieve effective colonization, invasion, and persistence inside the host and are increased by its innate tolerance to stress from the environment and other substances like antibiotics, disinfectants, and heavy metals [3]. Infections of burn wounds are regarded as a major health problem caused by bacterial germs colonizing burn injuries [4]. Burn wounds are thought to be a good place for opportunistic microorganisms of both foreign and endogenous origin to colonize when there has been substantial thermal injury [5]. Several factors, such as the type of burn injury itself, the patient's age, the extent of the injury, and the depth of the burn, along with microbial elements like the kind and quantity of organisms, the production of enzymes and toxins, aid in the colonization of the burn wound site and promote the spread of microorganisms that cause systemic dissemination [6]. Gram-positive, gram-negative bacteria and yeast from the host's normal upper respiratory and gastrointestinal flora as well as bacteria from the hospital environment invade burn wounds [7]. *P. aeruginosa* is regarded as the third leading cause of hospital-acquired infections accounted for about 18% of Urinary Tract Infections (UTIs) [8]. Infection in the urinary tract caused by *P. aeruginosa* can occur by either an ascending or descending route UTIs are clinically classified into complicated or uncomplicated infections. Complicated urinary tract infection is associate with different factors that either impaired the

urinary tract function or immune defence mechanism of the host, like urinary tract obstruction, immune suppression, kidney failure, kidney transplantation, pregnancy and formation of calculi [9]. Uncomplicated urinary tract infections typically occur in otherwise healthy individuals who do not have any underlying structural abnormalities or pre-existing conditions that could compromise the urinary tract [10].

Antibiotic resistance is a growing problem in developing countries since the overuse of antibiotics in humans. *Pseudomonas aeruginosa* exhibits resistance against several antibiotics, such as β-lactams, quinolones, and aminoglycosides [11]. *P. aeruginosa's* primary defence mechanisms against antibiotic assault can generally be divided into three categories: intrinsic, acquired, and adaptive resistance. Low outer membrane permeability, the development of efflux pumps that drive antibiotics out of the cell, and the synthesis of enzymes that render antibiotics inactive are the three components of *P. aeruginosa's* intrinsic resistance. *P. aeruginosa* can develop resistance by mutational alterations or horizontal transfer of resistance genes. *P. aeruginosa* develops biofilm as part of its adaptive resistance, which acts as a diffusion barrier to prevent the bacterium from being exposed to antibiotics [12].

Genomic identification using variable regions within the 16S gene for species-specific differentiation has been widely used for precise identification of a wide range of clinically relevant bacterial pathogens. This method is particularly valuable where phenotypic methods could not provide an adequate level of discrimination or gave discrepant results. Because other highly conserved genes have not received as much attention, 16S continues to be the most popular stable target for bacterial identification and genetic evolutionary investigations [13, 14].

Many strategies developed to overcome the increasing problems of multidrug resistant bacteria such as focusing on active compounds extracted from microorganisms [15] or plants which have medicinal properties and potential pharmaceutical [16, 17]. Mutations are heritable changes in the base sequence of DNA. Some mutations can be beneficial to an organism, but most are actually harmful because the mutation will often result in the loss of an important cellular function [18]. In the presence of certain mutagen, the rate of mutation can increase dramatically. Mutagenes are chemicals or radiation (such as X-rays or ultraviolet (UV) light) that alter the cellular genetic material, deoxyribonucleic acid, in a way that is irreversible and heritable (DNA) [19]. When damaged nucleotides are incorrectly incorporated into the DNA molecule, it can lead to the formation of mutations, as the genetic code is disrupted and errors are introduced into the sequence [20]. Direct UV absorption by DNA mostly causes pyrimidine dimers to form between nearby pyrimidines in a DNA strand. These dimmers were created when molecules' electrons were excited, causing additional bonds to form between nearby pyrimidines. This altered the structure of the DNA inside the cell, which interfered with DNA replication [21].

For the past 100 years, it has been established that UV radiation, especially UVC in the 200–280 nm range, is extremely germicidal. UVC radiation with a wavelength of 254 nm is frequently used to kill and inactivate a wide variety of microbiological organisms [22, 23]. As a result, it finds employment in an increasing range of applications, including as the food industry's processing of water and air, wound care, and the use of highly antimicrobial devices. UVC therapy is a viable supplementary therapy for chronic wounds infected with resistant bacteria since it has been shown to be very successful in killing bacteria without causing unacceptable damage to host tissue and improving wound healing in patients with acute wound infections [24] [25]. The aim of this study is to examine the mutagenic effect of UVC radiation on resistant bacteria isolated from different clinical cases such as burns, wounds and Urinary Tract Infection.

Materials and Methods

Isolation of bacteria

For isolation of *P. aeruginosa*, 65 samples were collected from patients suffered from burns, wounds and UTI infections in three hospitals in Baghdad during a period of 4 months, 8/2021 to 11/2021. Burns and wounds samples were taken by a sterile swap, while urine samples were put in a sterile container. Subsequently, a swab from these samples was streaked over MacConkey agar, nutrient agar, and blood agar plates. For the purpose of isolating *P. aeruginosa,* non-lactose fermenting colonies on MacConkey agar were sub cultured on Cetrimide agar and incubated at 37°C for 24 hours. Identification of bacteria was depended on Gram staining, VITEK2 system, also further identification of *P. aeruginosa* isolates was done by molecular diagnosis of bacteria using 16S rRNA.

Molecular detection of *P. aeruginosa* isolates by 16S rNA

Accurate and systematic identification of *Pseudomonas* is crucial to early detection of specific infections and to commence clinical intervention at the earliest opportunity. For this reason, DNA extraction for the genomic DNA was done using (Reagent Genomic DNA Kit, Promega, USA). The first step included harvesting of the bacterial cells and for this reason, the bacterial culture was transferred to 1.5 ml microcentrifuge tubes, centrifuged for 1 minute at 14–16 000 x g, and the supernatant was then discarded. The DNA extraction was completed as recommended by the manufacture protocol.

The DNA purity and concentration were measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The next step is DNA amplification, the primers used for the amplification is listed in table (1) [26]. As a stock solution, lyophilized primers were dissolved in nuclease-free water to a final concentration of 100 pmol / µl. The procedure for the PCR utilizing the thermocycler is set up and listed in table (2). A working solution of these primers was created by adding 10 µl of primer stock solution (stored at freezer -20 C) to 90 µl of nuclease-free water

Table 1: the primers were supplied by Macrogen Company in a lyophilized form

Primer Name	Seq.	Annealing Temp. (°C)	Product size (bp)
27F	5`-AGAGTTTGATCCTGGCTCAG-3`		
1492R	5`-TACGGTTACCTTGTTACGACTT-3`	**60**	**1500**

Table 2: PCR Program setup

Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	60	00:30	
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

Agarose gel electrophoresis was used to verify the existence of amplification following PCR. The Ethidium bromide-stained bands in gel were visualized using Gel imaging system. 1500-bp size amplicons were checked on electrophoresis gels to determine whether the 16S rRNA gene was present. Amplification of 16s RNA gene *P. aeruginosa* species was fractionated on 1.5% agarose gel electrophoresis stained with Ethidium Bromide.

PCR products were sent for Sanger sequencing using ABI3730XL, automated DNA sequences, by Macrogen Corporation – Korea and then the results were analyzed by BLASTN analysis utilizing the local database as well as the NCBI database to confirm that the bacteria were *P. aeruginosa*.

Test for antimicrobial susceptibility

According to Kirby-Baur standard technique, antimicrobial susceptibility testing was performed on thirty five isolates of *P. aeruginosa* against ten antibiotics

Selected isolated colonies of *P. aeruginosa* from nutrient agar were cultured in five millilitre of brain heart infusion broth. The cultures were then incubated overnight at 37°C, centrifuged at 5000 rpm for five minutes, diluted to match a 0.5 McFarland turbidity standard, which is equivalent to approximately 1.5x10 ^{8} cells/ ml. Mueller-Hinton agar, pH 7.2 was sterilized, then poured into Petri dishes at only 5 mm depth. A sterilized swab was immersed in the broth culture of isolates, gently removed any excess suspension ,then cultured on the Mueller-Hinton agar plate in many directions for uniform growth , left it to dry for about 5 minutes, then gently press each antibiotic disc to the agar with a flame-sterilized forceps ,the plates were incubated overnight at an incubation temperature 37°C before reading the results that compared with CLSI, 2020 standard value.

UV radiation-induced mutagenesis of the *P. aeruginosa* isolates

Mutagenesis for five *Pseudomonas aeruginosa* isolate P1, P2, P3, P4, and P5 by UV irradiation was done by subjecting a fresh culture of each bacterial isolate in four Petri dishes to UV radiation in a dark place by used the UV transilluminator. *P. aeruginosa* suspension was prepared at a concentration of 1.5 x 10^{8} CFU/ml in comparison to McFarland's turbidity tube of 0.5, then 0.1ml of bacterial suspension was cultured on cetrimide agar to detect the viability of bacteria before mutation (control) as shown in figure 4 A. For mutagenesis of *P. aeruginosa* isolates, 0.1 ml of bacterial suspension was added to each of four sterilized cetrimide agar plates, which were labelled M1 through M4. The tray measurement of irradiation was roughly (15 x 25) cm, and the distance between the UV source and the suspension in the plates that were exposed to the radiation was precisely 11 cm. under a sterile conditions, the plates were subjected to UV light for 30, 90, 180, and 360 seconds, respectively, at a wavelength of 254 nm and energy of 60 J/cm2. Bacterial cells were streaked in (4) mutated plates, three replicates were used for each mutation time, and then all plates were incubated at 37°C for 24 hours. The number of viable cells was counted (colony forming unit using colony counter) and the bacteria that survived UV exposure were considered as mutants. The same procedure of antibiotic sensitivity test was done for muted *P. aeruginosa* isolates at each exposure time (30, 90,180 and 360) second**,** and then compared the results for each antibiotic before and after mutation at different exposure time [27].

Results and Discussion

***Pseudomon*as *aeruginosa* isolates Isolation and Identification**

P. aeruginosa were detected on MacConkey agar; they grow as pale pink colonies with a diameter of 1-3 mm. Some isolates showed a mucoid appearance that was connected to the development of biofilms.

From a total of 65 samples were taken from burns, wounds and UTI infections, the findings from VITEK2 system revealed that 35 isolates (53.8%) were identified as *P. aeruginosa* while the remaining 30 isolates (46.2%) had belonged to *P. fluorescens*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Serratia marcescens* and *Acinetobacter baumannii*.

Microscopic appearance

P. aeruginosa was appeared as gram-negative bacilli, slender shaped, medium length, and non-sporulating.

Identification of *P. aeruginosa* isolates by 16SrNA

To optimize antibiotic therapy, direct bacterial pathogen detection from patient samples must be quick and accurate. The extracted genomic DNA templates were successfully employed as templates for the 16S rRNA PCR and the result of gel electrophoresis is described in Figure (1) verifying the 16S rRNA gene segments from each bacterial isolate.

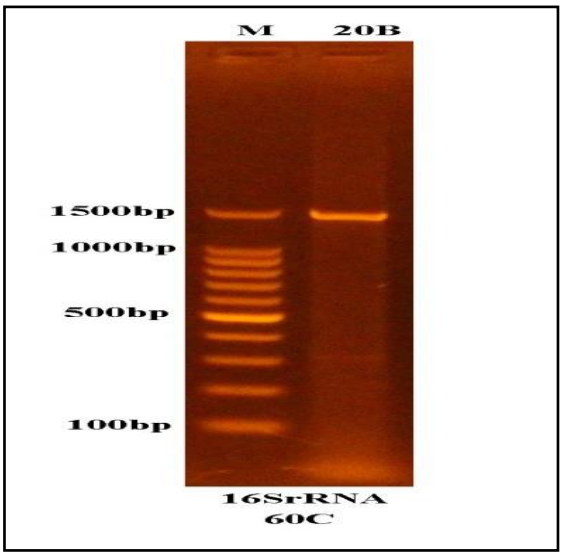

Figure 1: Results of the amplification of 16s RNA *gene P. aeruginosa* species was fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 20B resemble 1500bp PCR products

PCR products were sent for Sanger sequencing using ABI3730XL, automated DNA sequencer, by Macrogen Corporation – Korea. The results were received by email then analyzed using geneious software. Figure (2) displays the results of bacterial identification performed by BLASTN analysis utilizing the local database as well as the NCBI database. The bacteria were identified at the genus and species levels to be *P. aeruginosa*.

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Pseudomonas aeruginosa strain PAY 16S ribosomal RNA gene, partial sequence	2573	2573	100%	0.0	100.00%	MN700178.1
Pseudomonas aeruginosa strain LZS8436 16S ribosomal RNA gene, partial sequence	2573	2573	100%	0.0	100.00%	MF143547.1
Pseudomonas aeruginosa strain NA145 16S ribosomal RNA gene, partial sequence	2573	2573	100%	0.0	100.00%	KT005281.1
Pseudomonas aeruginosa strain NA137 16S ribosomal RNA gene, partial sequence	2573	2573	100%	0.0	100.00%	KT005274.1
Pseudomonas aeruginosa strain JQ-41 16S ribosomal RNA gene, partial sequence	2573	2573	100%	0.0	100.00%	KM948588.1
Pseudomonas sp. strain RG6 16S ribosomal RNA gene, partial sequence	2573	2573	100%	0.0	100.00%	ON221928.1
Bacterium AW5 16S ribosomal RNA gene, partial sequence	2573	2573	100%	0.0	100.00%	KF964356.1
Bacterium AW1(2013) strain AW1 16S ribosomal RNA gene, partial sequence	2573	2573	100%	0.0	100.00%	KF964355.1
Pseudomonas aeruginosa strain ALK320 16S ribosomal RNA gene, partial sequence	2573	2573	100%	0.0	100.00%	KC456535.1
Pseudomonas aeruginosa strain SXYC15 16S ribosomal RNA gene, partial sequence	2573	2573	100%	0.0	100.00%	JN999890.1
Pseudomonas aeruginosa strain HNYM11 16S ribosomal RNA gene, partial sequence	2573	2573	100%	0.0	100.00%	JN999889.1
Pseudomonas aeruginosa strain DSPV 005PSA 16S ribosomal RNA gene, partial sequence	2573	2573	100%	0.0	100.00%	JQ322234.1
Pseudomonas aeruginosa strain HK1-3 16S ribosomal RNA gene, partial sequence	2573	2573	100%	0.0	100.00%	JN661696.1
Pseudomonas aeruginosa strain S2QPS8 16S ribosomal RNA gene, partial sequence	2573	2573	100%	0.0	100.00%	HQ844502.1
Pseudomonas aeruginosa strain zxy0926 16S ribosomal RNA gene, partial sequence	2573	2573	100%	0.0	100.00%	HQ537785.1
Pseudomonas aeruginosa strain NA141 16S ribosomal RNA gene, partial sequence	2571	2571	99%	0.0	100.00%	KT005277.1

Figure 2: Data Analysis (BLAST results) using geneious software

Test for antibiotic susceptibility

The antibiotic susceptibility test results for 35 *P. aeruginosa* isolates demonstrated that 100% of isolates were sensitive to colistin and ciprofloxacin, while 31 (89%), 28 (80%) of isolates showed a sensitivity to gentamycin & Imipenem. On the other hand, the isolates were found resistant to piperacillin-tazobactum, cefotaxime and ceftazidime at high ratio (35) 100 %, 32 (91%), 30 (86%), respectively. Moderate resistance to Aztreonam, levofloxacin and ceftriaxone at percentage 26 (74%), 25 (71%), 22 (63%) respectively (figure 3).

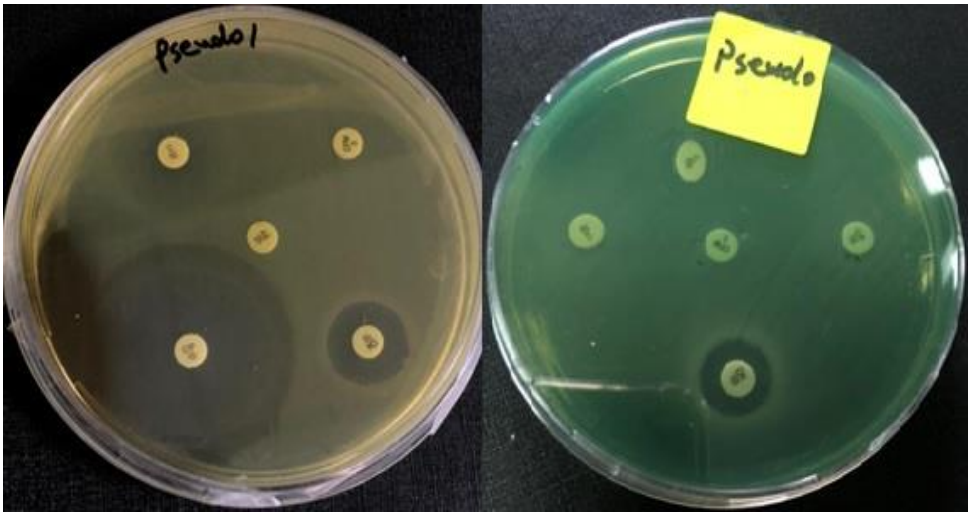

Figure 3: antibiotic sensitivity test f*or P. aeruginosa* isolates added information about the test Out of 35 tested *P. aeruginosa* isolates; five isolates were selected as they exhibited multidrug resistant against tested antibiotics.

Table 3: susceptibility Test for five multi drug resistant isolates of *P. aeruginosa*

Bacterial isolates	TZP mg/ml	CAZ mg/ml	LE mg/ml	CTR mg/ml	AZT mg/ml	CTX mg/ml	IPM mg/ml	CN10 mg/ml	CLM mg/ml	CIP5 mg/ml
P1	R	R	R	R	R	R	I	I	S	S
P2	R	R	R	R	R	R	I	I	S	S
P3	R	R	R	I	R	R	S	S	S	S
P4	R	R	R	R	R	R	R	I	S	S
P5	R	R	R	I	R	R	I	R	S	S

Note. S. sensitive, R .resistant, I .intermediate

***Pseudomonas aeruginosa* mutation**

Five multidrug resistant *Pseudomonas aeruginosa* isolates, P1, P2, P3, P4 and P5 isolates were selected for mutation by UV irradiation at various time periods (30, 90, 180, and 360 Sec). Results of *Pseudomonas aeruginosa* isolates mutation after exposure to UV irradiation from (30 - 360 sec) were close to each other but P1 isolates showed more response to UV irradiation in compares to other isolates, therefore it was selected as the best *Pseudomonas aeruginosa* isolates to study the effect of UV radiation on antibiotic susceptibility. There is a difference in response to UV radiation because this type of radiation cause random mutation that may affect different locus which might be on the resistance gene of some isolates while it affect other sites in the remaining isolates.

P. aeruginosa P1 isolate undergo mutation for various time periods (30, 90, 180, and 360 Sec) reduced its viability, and the number of viable cells count was changed from uncountable in control 4A to decrease number over time in M2 and M3 plates until reach to (60) x 10^8 how did you count this number the plates over crowded in M4 plate respectively as shown in figure 4 B, C, and D. Also, the isolate become more sensitive at ratio 40% toward the antibiotic's ciprofloxacin, colistin, gentamycin and Imipenem in which zones of inhibition recorded (27,21,18,19) in control isolate. After mutation, the inhibition zones increased to 37, 30, 28, and 25 mm, respectively, for each of the different time points examined. Additionally, *P. aeruginsa* P1 isolate showed changes in responses from resistant to susceptible towards three antibiotics Aztreonam, Cefotaxime, and Levofloxacin reaching to (25, 23 and 21 mm) zones of inhibition, respectively, while three antibiotics like Piperacillin-tazobactum, Ceftriaxone and Ceftazidime showed the same results before and after mutation as mention in table (4) and figure (4).

Table 4: Inhibition zone of mutated *P. aeruginosa* P1 before and after exposed to UV light for different exposure time

Antibiotics	**Mean of Inhibition zone (mm) Control**	**Mean Inhibition zone of mutated P1 (mm)**			
		M1	**M2**	**M3**	**M4**
Ciprofloxacin (CIP) 5 mg	27	28.6	31	36	37
Gentamicin (CN) 10 mg	18	20	21.6	23.3	28
Aztreonam (AZT) 30 mg	9.3	9.6	15.6	22.3	25
Cefotaxime (CTX) 30 mg	9.3	14.6	18.3	21	23
Piperacillin-tazobactum (TZP) 100/10 mg	8.3	8.3	8.3	9	9
Ceftriaxone (CTR) 30 mg	8	8	8.3	9	9
Colistin (CLM) 10 mg	21	22.3	24	25.3	30
Imipenem (IPM) 10 mg	19	20	21.6	22.6	25
Ceftazidime (CAZ) 30 mg	7	7.3	8	8	8
Levofloxacin (LE) 5 mg	9	9	14	18.3	21
L.S.D=0.05	**Antibiotics= 9.61; Mutation Time= 3.1 Antibiotics* Mutation Time.= 12.36**				

Note: M1….M4 mutation time (30, 90, 180 and 360 Second) respectively

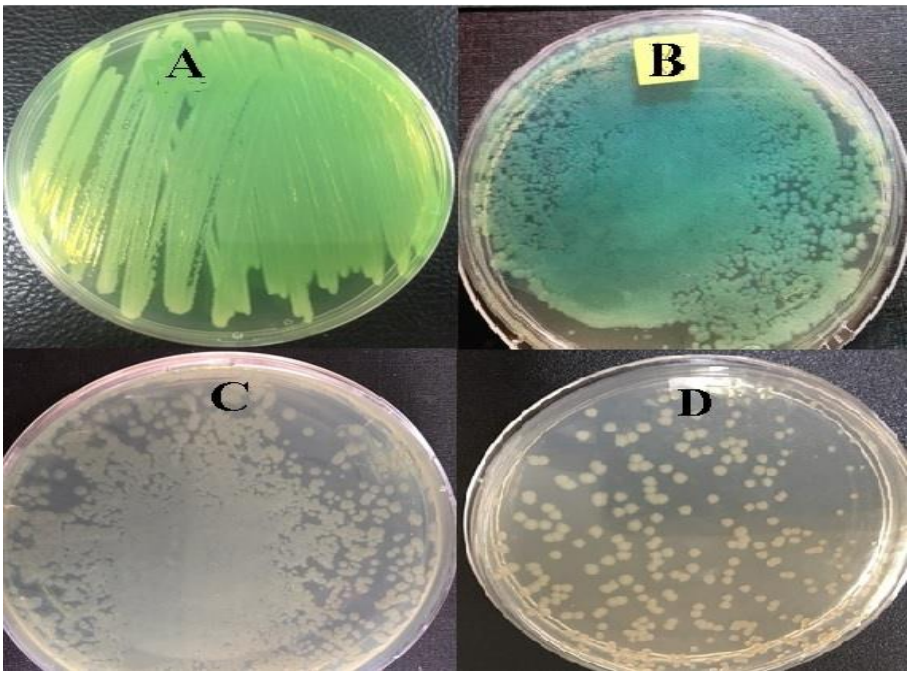

Figure 4: (A) Control *P. aeruginosa* P1, (B) mutated P1 isolates during 90 second, (C) mutated P1 isolates during 180 second, (D) mutated P1 isolates during 360 second

The antibiotic susceptibility test found a notable rise in the mean of inhibition zone for ciprofloxacin in relative to all other antibiotics types examined in the study. According to mutation time there was a significant increase in the mean inhibition zone in M4 in all antibiotics treatment except Piperacillin-tazobactum, Ceftriaxone, and Ceftazidime that inhibition zone recorded 9, 9 and 8 mm respectively.

The interaction between antibiotics and mutation time indicated that there was a significant increase in mean inhibition zone in M3 and M4 using ciprofloxacin antibiotics compared to all other interaction treatments tested. Because *P. aeruginosa* produces aminoacetophenone in vitro, it's frequently gives off a grape-like odour that can be recognized on nutrient agar. These findings matched those of woods *et al* [28].

A loopful of bacterial colony further streaked on Cetrimide agar then incubated at 37 °C for 24 hours to provide a conclusive diagnosis of *P. aeruginosa*. On Cetrimide agar plates, Pseudomonas isolates produce a blue-green pyocyanine colour. The colour of the pigment diffuses into the growing media. As noted by Cueva et al. in 2020, P. aeruginosa also developed pyoveridine pigments that fluoresced under UV light [29].

After a burn injury, burn wound infection was the main factor responsible for high morbidity and mortality. *P. aeruginosa*, *Staph. aureus*, and *Acinetobacter baumanni* infections are well-known pathogens that can grow in body fluids and seriously harm if septicemia developed that even kill patients [30]. The expression of typical virulence factors such as pyocyanin and pyoverdine was even enhanced virulence of *P. aeruginosa* in burn wound site [5]. Infections by *P. aeruginosa* at the Mutohari Burn Center in Tehran were examined in a study (1999 - 2000). During the course of the study, 230 bacterial strains and 190 positive cultures were identified from swabs or biopsy tissues. The majority of organisms were determined to be *Pseudomonas* (60%) with Acinetobacter (15%), *Staphylococcus aureus* (11%) and miscellaneous species (5%) following [12].

P. aeruginosa growth was stimulated in human burn wound exudates detected in patients prior to antibiotic therapy. While no other gram-negative bacterial growth such as *Acinetobacter* and *E. coli*, or Gram-positive bacteria like *S. aureus*, are isolated, suggest an inhibitory effect related to the expression levels of key Quorum sensing systems genes, *LasI/R*, and *RhlI/R* [31].

P. aeruginosa is causative agent of about 15% -25% of nosocomial infections like septicemia in intensive-care units [32]. *Pseudomonas aeruginosa* is considered as one of the most important causes of catheter-associated UTIs [33]. A study by Motbainor *etal*, (2020) who collected 200 urine specimens from patients with urethral catheters during the period from March 2020 to December 2021 and recorded that most predominant isolated bacterium was *Pseudomonas aeruginosa* followed by *E. coli* and *Proteus spp* [34].

It is well known that *P. aeruginosa* has a major impact on human health because of decrease activity of antibiotic, which results from the potent effects of several antibiotic efflux pumps combined with determinants of chromosomally mediated antibiotic resistance like mexAB, mexXY, oprM, and poor bacterial cellular envelope permeability to the antibacterial agent [35]. In South Africa at Memorial Children's Hospital, an evaluation of patients with clinically severe *P. aeruginosa* infections in the burns unit was conducted. 500 (16.6%) out of 3000 bacteriology samples collected over the course of the 37-month trial tested positive for *P. aeruginosa*. 90.1% of isolates were resistant to povidone-iodine, compared to 1.9% that were sensitive to chlorhexidine. Notably, Tobramycin exhibited the lowest resistance rate at 2.3%, whereas Piperacillin-Tazobactam showed the highest resistance rate at 35.8%, indicating significant differences in the susceptibility of the isolates to these antibiotics [36].

P. aeruginosa isolate that is multidrug resistant (MDR) which resistant to at least three antimicrobial classes, including carbapenem, fluoroquinolone, penicillin, cephalosporin, and aminoglycoside [5]. 65% of *P. aeruginosa* found to be multi drug resistant in one investigation. Ceftazidime and cefotaxime had the highest rates of antibiotic resistance, while imipenem, piperacillin, and gentamicin had the lowest rates [37]. The production of other enzymes, like metallo-β-lactamases (MBLs) and extended spectrum β-lactamases (ESBLs), that inactivate beta-lactams and carbapenem could be the mechanism of resistance in multidrug-resistant *P. aeruginosa* [38].

Changes in the chromosomally encoded determinants' genetic makeup or horizontal gene transfer can both cause antibiotic resistance can both cause *P. aeruginosa* to develop acquired resistance [4]. As a result of antibiotic therapy being hampered by difficult-to-treat resistant strains, MDR bacteria may cause illnesses [39]. Additionally, mobile genetic elements like integrons, plasmids, insertion sequences, transposons, and gene cassettes can spread resistance genes to other bacteria [40]. Later studies have shown genetically diverse strains of *Staphylococcus aureus are* circulating in burn wounds [41, 42] and bacteria with transmissible R-factors cause members of the *Enterobacteriaceae* family, *P. aeruginosa*, and *Vibrio cholera* to develop multiple antibiotic resistances [11]. Hyper mutation makes it easier for *P. aeruginosa* strains to develop antibiotic resistance, which leads to chronic infections [43].

One alternate method for selecting targeted mutations with the possibility of only single nucleotide changes is UV-based random mutagenesis [44]. Tetracycline and chloramphenicol resistance rates in the entire coliform population after UV irradiation were considerably lesser than those in the total coliform population before UV irradiation. This discovery was linked to the mechanism of R-factor-mediated tetracycline resistance [45]. Studies on DNA polymerase I (polyA)-deficient *P. aeruginosa* strains indicate that UV mutability in pMG2-containing bacteria may be caused by a plasmid-determined repair resynthesize function [46].

The lifespan of these isolates was improved by the presence 15 out of 34 distinct resistance (R) plasmids, according to research on *P. aeruginosa* subjected to ultraviolet irradiation (UV)

in wild-type, uvr, and polA mutants. The two most plasmids, pBS12 and pBS31, increased UV-induced mutability in the wild-type strain while shielding uvr+ strains from the lethal effect [47]. In a different investigation on *P. aeruginosa*, only 5 out of 26 strains were found to be resistant to the effects of UV irradiation. FP51 and FP59 are two of the plasmid FP factors, whereas R 910 is an R factor. UV mutagenesis is increased in the presence of these plasmids because they may contribute to the excision repair process' repair resynthesize stage [48]. The impact of R plasmid on radiation such as ultraviolet and gamma ray induced mutation in *P. aeruginosa* was investigated in strains carrying the revertible markers *hisO27* and *trpB1* as well as the conventional - sensitive markers *polA3* or *rec-2*. R plasmids *pPL1*, *R2*, and *pMG15* improved the ultraviolet radiation survival isolation and ultraviolet-induced mutation of mutant type in contrast to the lack of a R plasmid, where radiation-induced mutation was independent of the polA+ genotype and dependent on the recA+ genotype [49].
UVR affects skin cells differently biologically because the skin is composed of layers that are vary in depth and have unique physical and chemical characteristics. The type of skin has a major influence on the minimal erythema dose (MED). The induction of minimal erythema dose response curves demonstrates that human skin is more sensitive to UV-C light at 254 nm than to UV-B radiation at 300 nm. This short wavelength of UV-C has a limited (superficial) influence on the epidermis, which may be linked to long-term and random effects on the skin. The effects on the skin also rely on the duration of exposure, and in our work, we employed a very little exposure period (in seconds) to bacteria [50].
A study by Wang et al comprehensively evaluated the effect of the UV/chlorine on *Pseudomonas aeruginosa* as the target microorganism. The level of toxic *opr* gene in *P. aeruginosa* decreased by more than 99% after UV/chlorine treatment [51]. Also Argyraki et al test the inactivation efficiency of two different light-based treatments, namely ultraviolet B (UVB) and ultraviolet C (UVC) irradiation, on *Pseudomonas aeruginosa* biofilms at different growth stages. UVC irradiation induced inactivation on mature biofilms [52].

Conclusions

Although P. aeruginosa strains were universally susceptible to colistin and ciprofloxacin, they exhibited resistance to several other antibiotics, especially piperacillin-tazobactam, cefotaxime, and ceftazidime. Exposure to UV radiation caused a change in susceptibility patterns in *P. aeruginosa*. Mutations induced by UV radiation can impact resistance profiles potentially offering benefits or aiding in understanding resistance mechanisms better. Overall, this study underscores the importance of integrating molecular diagnostics with traditional microbiological techniques for comprehensive pathogen characterization and the need for innovative approaches to manage antibiotic resistance in clinical settings.

Data Availability: All data are available upon request from the corresponding author.
Conflicts of Interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

References

[1] N. Othman, M. Babakir-Mina, C. K. Noori, and P. Y. Rashid, "Pseudomonas aeruginosa infection in burn patients in Sulaimaniyah, Iraq: risk factors and antibiotic resistance rates," (in eng), *The Journal of Infection in Developing Countries,* vol. 8, no. 11, pp. 1498-502, Nov 13 2014, doi: 10.3855/jidc.4707.

[2] N. El Hamzaoui, A. Barguigua, S. Larouz, and M. Maouloua, "Epidemiology of burn wound bacterial infections at a Meknes hospital, Morocco," *New Microbes and New Infections,* vol. 38, p. 100764, 2020.

[3] R. Bryant and D. Nix, *Acute and chronic wounds: current management concepts*. Elsevier Health Sciences, 2015.

[4] W. a. Al-Daraghi and M. Al-Badrwi, "Molecular Detection for Nosocomial Pseudomonas aeruginosa and its Relationship with multidrug Resistance, Isolated from Hospitals Environment," *Medico Legal Update,* vol. 20, no. 1, pp. 631-636, 04/09 2020, doi: 10.37506/mlu.v20i1.433.

[5] A. A. Ahmed, N. A. Juyee, S. A. Hasan, and M. Z. Abedin, "Microbiological Screening and Antimicrobial Sensitivity Profiling of Wound Infections in a Tertiary Care Hospital of Bangladesh," *European Journal of Medical and Health Sciences,* vol. 2, no. 5, pp. 101-106, 2020.

[6] I. A. Hussien, K. A. Habib, and K. A. Jassim, "Bacterial colonization of burn wounds," *Baghdad Science Journal,* vol. 9, no. 4, pp. 623-631, 2012.

[7] E. Coetzee, H. Rode, and D. Kahn, "Pseudomonas aeruginosa burn wound infection in a dedicated paediatric burns unit," *South African Journal of Surgery,* vol. 51, no. 2, pp. 50-53, 2013.

[8] G. Maharjan, P. Khadka, G. Siddhi Shilpakar, G. Chapagain, and G. R. Dhungana, "Catheter-associated urinary tract infection and obstinate biofilm producers," *Canadian journal of infectious diseases and medical microbiology,* vol. 2018, 2018.

[9] B. H. Saleh, R. N. Ibrahim, H. E. Abdul-Wahed, and Z. T. Salih, "Study the antibacterial activity of green tea leaves Camellia sinensis ethanolic extract against different pathogenic bacteria isolated from urinary tract infections," *Plant Archives,* vol. 20, no. 1, pp. 1077-1084, 2020.

[10] G. Jiménez-Guerra *et al.*, "Urinary tract infection by Acinetobacter baumannii and Pseudomonas aeruginosa: evolution of antimicrobial resistance and therapeutic alternatives," *Journal of medical microbiology,* no. 6, pp. 790-797, 2018.

[11] E. Sanganyado and W. Gwenzi, "Antibiotic resistance in drinking water systems: Occurrence, removal, and human health risks," *Science of The Total Environment,* vol. 669, pp. 785-797, 2019.

[12] H. K. Estahbanati, P. P. Kashani, and F. Ghanaatpisheh, "Frequency of Pseudomonas aeruginosa serotypes in burn wound infections and their resistance to antibiotics," *Burns,* vol. 28, no. 4, pp. 340-348, 2002.

[13] D. L. Church, L. Cerutti, A. Gürtler, T. Griener, A. Zelazny, and S. Emler, "Performance and application of 16S rRNA gene cycle sequencing for routine identification of bacteria in the clinical microbiology laboratory," *Clinical microbiology reviews,* vol. 33, no. 4, pp. e00053-19, 2020.

[14] Z. Ghazi Faisal, M. Sallal Mahdi, and K. H. Alobaidi, "Optimization and Chemical Characterization of Biosurfactant Produced from a Novel Pseudomonas guguanensis Strain Iraqi ZG. KM," *International Journal of Microbiology,* vol. 2023, 2023.

[15] M. S. Mahdi, H. K. Ibrahim, and D. N. Al-ugaili, "Antimicrobial Activity of Geobacillus thermoleovorans Ir1 Active Compounds against Pathogenic Bacteria," *Iraqi Journal of Science,* 2024, doi: https://doi.org/10.24996/ijs.2024.65.3.10.

[16] J. R. Al-Obaidi, B. S. Al-Taie, M. Y. Allawi, and K. H. Al-Obaidi, "Multiple-Usage Shrubs: Medicinal and Pharmaceutical Usage and Their Environmental Beneficiations," in *Medicinal and Aromatic Plants: Healthcare and Industrial Applications*, T. Aftab and K. R. Hakeem Eds. Cham: Springer International Publishing, 2021, pp. 445-484.

[17] S. A. Aljubouri and K. H. Alobaidi, "Assessment of the Antivirulence Potential of *Tamarix aphylla* Ethanolic Extract against Multidrug-Resistant *Streptococcus mutans* Isolated from Iraqi Patients," *The Scientific World Journal,* vol. 2023, p. 6662262, 2023/08/04 2023, doi: 10.1155/2023/6662262.

[18] M. B. Said, S. Khefacha, L. Maalej, I. Daly, and A. Hassen, "Effect of ultraviolet, electromagnetic radiation subtype C (UV-C) dose on biofilm formation by Pseudomonas aeruginosa," *African Journal of Microbiology Research,* vol. 5, no. 25, pp. 4353-4358, 2011.

[19] B. Caballero, L. Trugo, and P. Finglas, *Encyclopedia of food sciences and nutrition: Volumes 1-10* (no. Ed. 2). Elsevier Science BV, 2003.

[20] B. H. Saleh, R. N. Ibrahim, and R. M. Ibrahim, "Study the Mutagenic Effects of Ultraviolet (UV) on Viability and Antibiotic Sensitivity of Enteropathogenic *Escherichia coli* Isolated from Infants Diarrhea," *World Journal of Pharmaceutical Research,* Research Article vol. 4, no. 7, p. 13, 2015 2015.

[21] A. K. Banaś, P. Zgłobicki, E. Kowalska, D. Dziga, and W. Strzałka, "All you need is light. Photorepair of UV-induced pyrimidine dimers," *Genes,* vol. 11, no. 11, p. 1304, 2020.

[22] H. Singh, S. K. Bhardwaj, M. Khatri, K.-H. Kim, and N. Bhardwaj, "UVC radiation for food safety: An emerging technology for the microbial disinfection of food products," *Chemical Engineering Journal,* vol. 417, p. 128084, 2021/08/01/ 2021, doi: https://doi.org/10.1016/j.cej.2020.128084.

[23] B. H. Saleh, R. N. Ibrahim, and D. N. Al-Ugaili, "The Effect of Diode Laser on Viability and Antibiotic Sensitivity of Streptococcus mutans Isolated From Dental Caries," *Iraqi Journal of Science,* pp. 583-593, 2023.

[24] N. A. Aleem, M. Aslam, M. F. Zahid, A. J. Rahman, and F. U. Rehman, "Treatment of Burn Wound Infection Using Ultraviolet Light: A Case Report," *Journal of the American College of Clinical Wound Specialists,* vol. 5, no. 1, pp. 19-22, 2013/04/01/ 2013, doi: https://doi.org/10.1016/j.jccw.2014.07.002.

[25] S. Marasini, A. C. Zhang, S. J. Dean, S. Swift, and J. P. Craig, "Safety and efficacy of UV application for superficial infections in humans: A systematic review and meta-analysis," *The Ocular Surface,* vol. 21, pp. 331-344, 2021/07/01/ 2021, doi: https://doi.org/10.1016/j.jtos.2021.03.002.

[26] N. M. Ali, S. Chatta, I. Liaqat, S. A. Mazhar, B. Mazhar, and S. Zahid, "Pseudomonas aeruginosa associated pulmonary infections and in vitro amplification virulent rhamnolipid (rhlR) gene," *Brazilian Journal of Biology,* vol. 82, 2020.

[27] F. D. Halstead *et al.*, "Antibacterial activity of blue light against nosocomial wound pathogens growing planktonically and as mature biofilms," *Applied and environmental microbiology,* vol. 82, no. 13, pp. 4006-4016, 2016.

[28] S. J. Wood, T. M. Kuzel, and S. H. Shafikhani, "Pseudomonas aeruginosa: infections, animal modeling, and therapeutics," *Cells,* vol. 12, no. 1, p. 199, 2023.

[29] A. R. Cueva, O. Pham, A. Diaby, D. Fleming, K. P. Rumbaugh, and G. E. Fernandes, "Pyoverdine assay for rapid and early detection of Pseudomonas aeruginosa in burn wounds," *ACS Applied Bio Materials,* vol. 3, no. 8, pp. 5350-5356, 2020.

[30] K. M. Ali and B. M. A. Al-Jaff, "Source and antibiotic susceptibility of gram-negative bacteria causing superficial incisional surgical site infections," *International Journal of Surgery Open,* vol. 30, p. 100318, 2021/03/01/ 2021, doi: https://doi.org/10.1016/j.ijso.2021.01.007.

[31] M. R. Gonzalez *et al.*, "Effect of human burn wound exudate on Pseudomonas aeruginosa virulence," *Msphere,* vol. 1, no. 2, pp. e00111-15, 2016.

[32] Noha A Hassuna, Amany Hosney Ibrahim Mohamed, Sahar Mohamed Abo- Eleuoon, and H. A.-W. A. Rizk, "High Prevalence of Multidrug Resistant Pseudomonas aeruginosa Recovered from Infected Burn Wounds in Children," *Archives of Clinical Microbiology,* vol. 6, no. 4, p. 7, 2015.

[33] A. Flores-Mireles, T. N. Hreha, and D. A. Hunstad, "Pathophysiology, treatment, and prevention of catheter-associated urinary tract infection," *Topics in spinal cord injury rehabilitation,* vol. 25, no. 3, pp. 228-240, 2019.

[34] H. Motbainor, F. Bereded, and W. Mulu, "Multi-drug resistance of blood stream, urinary tract and surgical site nosocomial infections of Acinetobacter baumannii and Pseudomonas aeruginosa among patients hospitalized at Felegehiwot referral hospital, Northwest Ethiopia: a cross-sectional study," *BMC infectious diseases,* vol. 20, pp. 1-11, 2020.

[35] D. K. Neyestanaki, A. Mirsalehian, F. Rezagholizadeh, F. Jabalameli, M. Taherikalani, and M. Emaneini, "Determination of extended spectrum beta-lactamases, metallo-beta-lactamases and AmpC-beta-lactamases among carbapenem resistant Pseudomonas aeruginosa isolated from burn patients," *Burns,* vol. 40, no. 8, pp. 1556-1561, 2014.

[36] E. C. Moore, D. V. Pilcher, M. J. Bailey, H. Stephens, and H. Cleland, "The Burns Evaluation and Mortality Study (BEAMS): Predicting deaths in Australian and New Zealand burn patients admitted to intensive care with burns," *Journal of Trauma and Acute Care Surgery,* vol. 75, no. 2, pp. 298-303, 2013, doi: 10.1097/TA.0b013e318295409d.

[37] D. Shokri *et al.*, "Determination of Acquired Resistance Profiles of Pseudomonas aeruginosa Isolates and Characterization of an Effective Bacteriocin-Like Inhibitory Substance (BLIS) Against These Isolates," (in eng), *Jundishapur journal of microbiology,* vol. 9, no. 8, p. e32795, Aug 2016, doi: 10.5812/jjm.32795.

[38] K. S. M. Azab, M. A. Abdel-Rahman, H. H. El-Sheikh, E. Azab, A. A. Gobouri, and M. M. S. Farag, "Distribution of Extended-Spectrum β-Lactamase (ESBL)-Encoding Genes among Multidrug-Resistant Gram-Negative Pathogens Collected from Three Different Countries," *Antibiotics,* vol. 10, no. 3, p. 247, 2021. [Online]. Available: https://www.mdpi.com/2079-6382/10/3/247.

[39] M. Vahdani, L. Azimi, B. Asghari, F. Bazmi, and A. Rastegar Lari, "Phenotypic screening of extended-spectrum ß-lactamase and metallo-ß-lactamase in multidrug-resistant Pseudomonas aeruginosa from infected burns," (in eng), *Annals of burns and fire disasters,* vol. 25, no. 2, pp. 78-81, Jun 30 2012.

[40] P. Owlia, H. Saderi, S. Mansouri, S. Salemi, and H. Ameli, "Drug resistance of isolated strains of Pseudomonas Aeruginosa from burn wound infections to selected antibiotics and disinfectants," *Iranian Journal of Pathology,* vol. 1, no. 2, pp. 61-64, 2006. [Online]. Available: https://ijp.iranpath.org/article_8903_8f90f31b274a559e618040b12f4e9a18.pdf.

[41] M. Goudarzi *et al.*, "Genetic diversity of methicillin resistant Staphylococcus aureus strains isolated from burn patients in Iran: ST239-SCCmec III/t037 emerges as the major clone," *Microbial Pathogenesis,* vol. 105, pp. 1-7, 2017/04/01/ 2017, doi: https://doi.org/10.1016/j.micpath.2017.02.004.

[42] M. Navidinia, H. Goudarzi, R. Pouriran, H. Azimi, and M. Goudarzi, "Molecular Analysis and Integron Carriage of Mupirocin-Resistant Staphylococcus aureus Strains Isolated from Burn Wound Infections, Tehran, Iran," *Archives of Clinical Infectious Diseases,* Research Article vol. 14, no. 1, p. e84764, 2019, doi: 10.5812/archcid.84764.

[43] I. H. Igbinosa, A. Beshiru, and E. O. Igbinosa, "Antibiotic resistance profile of Pseudomonas aeruginosa isolated from aquaculture and abattoir environments in urban communities," *Asian Pacific Journal of Tropical Disease,* vol. 7, no. 1, pp. 47-52, 2017.

[44] J. L. Bose, "Chemical and UV mutagenesis," *The Genetic Manipulation of Staphylococci: Methods and Protocols,* pp. 111-115, 2016.

[45] M. C. Meckes, "Effect of UV light disinfection on antibiotic-resistant coliforms in wastewater effluents," *Applied and environmental microbiology,* vol. 43, no. 2, pp. 371-377, 1982, doi: doi:10.1128/aem.43.2.371-377.1982.

[46] P. Lehrbach, A. H. C. Kung, B. T. O. Lee, and G. A. Jacoby, "Plasmid Modification of Radiation and Chemical-mutagen Sensitivity in Pseudomonas aeruginosa," *Microbiology,* vol. 98, no. 1, pp. 167-176, 1977, doi: https://doi.org/10.1099/00221287-98-1-167.

[47] C. N. H. Marques, V. C. Salisbury, J. Greenman, K. E. Bowker, and S. M. Nelson, "Discrepancy between viable counts and light output as viability measurements, following ciprofloxacin challenge of self-bioluminescent Pseudomonas aeruginosa biofilms," *Journal of Antimicrobial chemotherapy,* vol. 56, no. 4, pp. 665-671, 2005, doi: 10.1093/jac/dki285.

[48] V. Krishnapillai, "Resistance to ultraviolet light and enhanced mutagenesis conferred by Pseudomonas aeruginosa plasmids," *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* vol. 29, no. 3, pp. 363-371, 1975/09/01/ 1975, doi: https://doi.org/10.1016/0027-5107(75)90057-3.

[49] P. R. Lehrbach, B. T. Lee, and C. D. Dirckze, "Effect of repair deficiency and R plasmids on spontaneous and radiation-induced mutability in Pseudomonas aeruginosa," *Journal of Bacteriology,* vol. 139, no. 3, pp. 953-960, 1979, doi: doi:10.1128/jb.139.3.953-960.1979.

[50] SCHEER, "(Scientific Committee on Health, Environmental and Emerging Risks), Committee on Health, Environmental and Emerging Risks), Opinion on Biological effects of UV C radiation relevant to health with particular reference to UV C lamps, 2 February 2017," ed, 2021.

[51] L. Wang *et al.*, "Assessment of the UV/Chlorine Process in the Disinfection of Pseudomonas aeruginosa: Efficiency and Mechanism," *Environmental Science & Technology,* vol. 55, no. 13, pp. 9221-9230, 2021/07/06 2021, doi: 10.1021/acs.est.1c00645.

[52] A. Argyraki, M. Markvart, L. Bjørndal, T. Bjarnsholt, and P. M. Petersen, "Inactivation of Pseudomonas aeruginosa biofilm after ultraviolet light-emitting diode treatment: a comparative study between ultraviolet C and ultraviolet B," *Journal of Biomedical Optics,* vol. 22, no. 6, p. 065004, 2017. [Online]. Available: https://doi.org/10.1117/1.JBO.22.6.065004.