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Research Article

Anticancer activity of pyoverdine (PVD) producing by antibiotic-resistant *Pseudomonas aeruginosa* isolated from burn and wound infections

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Abstract
Pseudomonas aeruginosa can cause diseases and multidrug resistance. It can produce many pigments, such as pyoverdine, which has anticancer properties. Cancer is still a major issue in medical science. Therefore, this study aimed to use highly efficient alternative treatments, such as pyoverdine**.** One hundred fifty samples of burns and wounds were collected from patients hospitalized in BabylIt hospitals. All the isolates were cultivated on various media to identify all specimens, including blood agar, MacConkey agar, and cetrimid agar. The isolates were tested for antibiotic susceptibility. *P. aeruginosa* was cultured in Luria-Bertani (LB) medium to stimulate its production of PDV. Congo red method and microtiter plate were used to determine biofilm production. The findings showed 50 isolates of *P. aeruginosa* were dispersed among patients, 35/50 (70%) burns and 15/50 (30%) wound infections and only four of the 50 isolates produced PVD. *P. aeruginosa* was examined against 17 common antibiotics(Aztreonam, Ceftazidime, Cefepime, Cefriaxone, Piperacillin_ tazobactam, Piperacillin, Gentamicin, Tobramycin**,** Netilmicin**,** Amikacin, Ciprofloxacin, Norfloxacin, Gatifloxacin, Levofloxacin, Imipenem, Doripenem, Meropenem)and the majority of isolates exhibited MDR. By using the Congo red method out of 50, 4(8%) isolates gave a positive ability to form biofilm as a qualitative method. Among 4 isolates, ps1 and ps4 were more productive, so it was tested to complete the study. PVD had anticancer activity against two types of cell lines: Lung cancer cells (A549) with inhibition range from 31.800in 400 µg/ml to 7.200 %in 25 µg/ml and skin cancer cells (A375) with cell vitality range from 55.600in 400 µg/ml to 8.533% in 25 µg/ml.

Keywords: Biofilm **,** Cancer cell lines, Congo red , *Pseudomonase aeruginosa* , Pyoverdine (PVD), Succinic acid medium

INTRODUCTION

Pseudomonase aeruginosa is a very common bacteria that causes multiple infections in humans and animals. It can develop multiple resistance to antibiotics and cause hospital infections in hospitalized patients or healthy companions. *P. aeruginosa* causes infections in wounds and burns, as it possesses many virulent factors that enable it to invade and infect the host (Moradali *et al* .,2017). *P. aeruginosa* can form a biofilm, which is a way to resist unfavorable conditions, including resistance to antibiotics (Moradali *et al*.,2017). *P.aeruginosa* can produce and secrete many pigments that express virulence factors and cause pathogenicity in bacteria, including include pyocyanin and pyoverdine **(**Sen *et al*.,2019). Pyoveridines are chelated compounds that can seize iron and form complex compounds with it, as they are considered an iron carrier (Abbas *et al*.,2018). Pyoveridine produced by *P. aeruginosa* has high antibacterial and anticancer activity when produced in the presence of iron or free non-iron, and its effectiveness as an antibacterial is due to its ability to stimulate iron compounds (oradali *et al*.,2017). Cancer is still a major issue in medical science, especially with increasing death cases every year worldwide. Therefore, the present study aimed i) to use treatments with high efficiency, selectivity and less toxicity as the main goal in fighting the disease, ii) to test the ability of *P. aeruginosa* isolated from burns and wounds to produce pyoprevidin and test its ability to form biofilm and resist antibiotics and iii) to test the ability of pyoprevidin as the anticancer agent to solve the

problem of cancer as the more dangerous disease.

MATERIALS AND METHODS

Collection of samples

During the presented study, 150 swabs were collected from burn injuries and wounds from patients hospitalized in Imam al-Sadiq Hospital in Hilla in Babylon from August 2023 to November 2023.

All specimens were cultured on different media for identification of *Pseudomonas* such as blood agar, MacConkey agar, and cetrimid agar, using a sterile loop spread on the surface of agar media and incubated at 37 C° for 24 hr. Purified colonies were kept in nutrient broth containing glycerol at -20 C° . After final diagnosis of samples, 50 (33.3%) isolates of *P. aeruginosa* (named Pa1 to Pa50) were obtained,

After the growth of bacteria on MacConkey agar, blood agar, cetrimid agar and nutrient agar, their shape, size, texture, and colony arrangement were observed. A single colony was picked up stained with Gram stain and biochemical tests Catalase test, IMVC test, Oxidase test, Urease test, Triple sugar iron (TSI) test and Motility test were performed for identification of *Pseudomonas*(AL-Rubaye *et al*.,2015; Brown *et al*.,2017).

Antimicrobial susceptibility test

Antimicrobial susceptibility test was performed according to clinical laboratory guidelines (CLSI-2023) (0.5 McFarland tube was used to obtain 1.5×10^8 CFU/mL bacterial culture). The antibiotics and the disks used in the study are shown in Table 1.

Pseudomonas aeruginosa was grown in L B medium in 250 ml flakes for 1-5 days at a temperature of 37°C. The bacteria were monitored during the incubation period and the color change was monitored, as the appearance of a yellowish-green color is evidence of pyoverdine production, as indicated in Fig 1. To confirm production of PDV, it was grown on King A, which stimulates the production of pyocyanin and on King B agar, which stimulates the production of PDV (Cappuccino and Welsh 2018; Chandra *et al*.,2017) as indicated in Fig 2**.** The number of bacterial cells was determined by counting the number of CFU per ml.

After the color change appeared in both cases, the bacterial culture was centrifuged as a first step, after which it was filtered using a 0.2 micrometer microfilter to get rid of cell debris. To extract PVD from liquid media filtered through filter paper and to obtain PVD, equal amounts of chloroform were added, volume/ volume, then shaken quietly for an hour to get two layers. The upper brown layer was discarded, while the lower layer, yellowish-green, was kept. The process was repeated more than once to obtain pyoverdine free of impurities or cellular residues. After that, it was dried in an oven at 50 C° to evaporate the chloroform and concentrate the PVD (El-Fouly *et al.,* 2015).

Pyoverdine (PVD) concentration measurement

A Spectrophotometer was used to measure the absorption of PVD solution at a wavelength of 405 nm, and the concentration of PVD was ascertained using the following formula (Esraa *et al*.,2023).

The PVD concentration (µg/ml) equals (O.D405) × 17.072. ……Eq. 1

Where: optical density- OD.

Production of biofilms

The production of biofilms was qualitatively assessed using the Congo Red technique. The outcome of Congo red agar was noted as a colony's color changing from red to black.

The medium was prepared according to the manufacturer's instructions. The results were read after an incu-

Table 1. Antibiotics and antimicrobial disks used to determine antimicrobial sensitivity testing

Antibiotics	Antibiotics Classes	Abbreviation	µg / disk	Company/ origin
Aztreonam	Monobactams	ATM	30	India/Himedia
Ceftazidime		CAZ	30	Roseto /Italy
Cefepime	Cephems	CEP	30	Roseto /Italy
Cefriaxone		CIP	30	
Piperacillin tazobactam	β-Lactams combinations	PTZ	100/10	MAST/U.K
Piperacillin	Penicillins	PRL	100	Roseto /Italy
Gentamicin		CN	10	Condalab/Spain
Tobramycin		TOB	5	Tur/ Bioanalyse
Netilmicin	Aminoglycosi des	NET	30	Roseto /Italy
Amikacin		AK	30	Roseto /Italy
Ciprofloxaci N		CIP	5	Himedia/ India
Norfloxacin	Fluoroquinolon es	NX	10	Roseto /Italy
Gatifloxacin		GAT	5	Roseto /Italy
Levofloxacin		LEV	5	Roseto /Italy
Ofloxacin		OFX	5	Bioanalyse/Turkey
Imipenem	Carbepenem	IPM	10	Roseto /Italy
Doripenem		DOR	10	Bioanalyse /Turkey
Meropenem		MEM	30	Bioanalyse /Turkey

bation period of 24-48 hours at a temperature of 37 C˚ . If the colonies appeared black, this is evidence of biofilm production, but if they appeared red, this indicates that they are not producing biofilm (Forbes *et al.,*2017)
Microtiter plate (MTP) is a quantitative method to determine biofilm production by microtiter plate reader as described by (Jassim *et al*.,2023).
Isolates were classified as follows :
(ODc < OD < 2×ODc)…….. Weakly-adherent.
(2×ODc < OD < 4×OD)……. Moderately-adherent.
(4×OD < OD)…… Strongly-adherent.

Cytotoxicity effect

The cytotoxicity method was performed to investigate the possible cytotoxic effect of PVD against Lung cancer cells (A549) and skin cancer cells (A375) . These cancer cells were suspended in full RPMI1640 media and propagated for 24 hours in culture flasks at 37°C in a humid environment with an addition of 5% CO2. The growing medium was withdrawn and the sticky cells were rinsed twice with PBS solution once they had attained 80% confluency. Trypsin-EDTA solution (two to three mL) was added to the flask, and the flask was gently shaken and flipped over to cover the monolayer completely. The cells were allowed to separate from the flask surface by incubating the flask at 37°C for 1-2 minutes. By adding a complete RPMI-1640 medium, the trypsin was rendered inactive. Next, the cell suspension was transferred to flasks containing new complete RPMI medium. Cultured flasks were kept in an incubator with 5% ambient CO2 at 37°C. The requisite cell concentration was obtained using the trypan exclusion cell counting method by combining a volume of cell suspension with a volume of trypan blue stain. The cells were counted using a hemocytometer and the following formula after waiting for 3 minutes **(**Jawetz *et al.*,2019**)**

Total Cell Count mL-1 = Cell count x Dilution Factor (Sample Volume) x 10^4 ...Eq. 2

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay

MTT assay was used to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity and this test was conducted as described by (Kamali *et al*., 2020).

Ethical approval

The Helsinki Declaration's ethical guidelines were followed when conducting the study. The patient's verbal and analytical consent was obtained before any sample was taken. A local ethics commission examined and approved the study protocol, subject information, and permission form number M220109 on January 2, 2024.

RESULTS

The results of the isolation of different clinical samples (150) were distributed as burn infection patients 90 (60%) and wound infection patients 60 (40%) . The results revealed a high percentage of *P. aeruginosa*.50 were isolates distributed as burn infection patients 35/50 (70%) and 15/50 (30%) wound infection (table 1) and from the 50 isolates, only four isolates produced the PVD.
The morphological characteristics of colonies were examined on culture mediums (blood agar, MacConkey agar, and cetrimide agar). The colonies on the blood agar were sticky-textured, white to grey or dark-coloured bacteria that could hemolyze blood. While *P. aeruginosa* on Macconkey agar was a pale color due to its inability to ferment the lactose sugar present in this culture medium, it smelled similar to fermented grapes.

Table 3. Types, numbers, isolates and percentage of clinical samples collected from the patients

Clinical samples	No. of samples	No. of P.aeruginosa isolates	Percentage
Burns	90(60%)	35/50	70%
Wounds	60(40%)	15/50	30%
Total	150 (100%)	50	100%

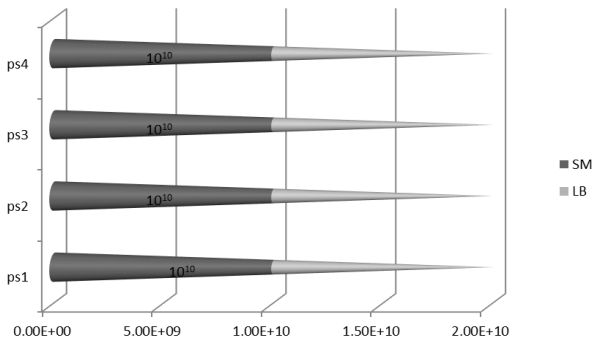


Fig 1. *Number of cells for the isolates that showed the ability to produce pyoverdine*

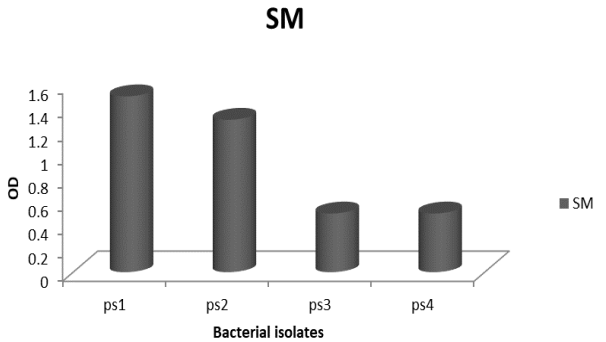


Fig. 2. *Isolates that could produce pyoverdin in liquid SM medium*

Table 4. Tests using biochemistry to identify isolates of *Psuedomonas aeruginosa*

Test	Results
Gram-stain	+
Catalase test ,	+
iOxidase test	+
H2S iproduction	-
iIndole test	-
iKligler's iron iagar	Alkaline / Alkaline
iMethyl-red	-
Voges-Proskauer	-
Pigments production	+
Urease	-
Simmon's citrate	+
Motility	+

Abbreviations : (+), positive test; (-), Negative test

The qualitative biochemical tests as listed in Table 4 were performed for more confirmation of *P.aeruginosa* isolates.

Antibiotic susceptibility testing was conducted for 50 isolates of Pseudomonas aeruginosa (Pa1-Pa50) against 17 types of antibiotics. The isolates showed resistance to most of the antibiotics used, especially the beta-lactam group. The highest resistance rate was to the antibiotics tobramycin, piperacillin, cefepime, imipenem, ofloxacin, aztreonam, and netilmicin, at 100%, as shown in Table 5. While the antibiotics showed norfloxacin (40%), piperacillin-tazobactam (13%), levofloxacin (21%), amikacin (35%), meropenem (21%), ciprofloxacin (42%) and doripenem (19%). Table 5 shows the phenotypic of antibiotic susceptibility of bacterial isolates in this study

It was found that the number of cells for the isolates that showed the ability to produce PVD was $1x10^{10}$, as shown in Fig. 1. PVD production was measured as the OD405 of culture supernatants. As expected, a difference was observed in culture broth color or growth capacity between *P. aeruginosa* cultured in liquid SM broth media. The optical density at 405 nm (OD_{405}) values suggested that these strains could produce PVD in a liquid medium, as shown in Fig. 2**.** The present study showed that (4\50) 8 % of isolates were a producer for PVD siderophore as shown in Fig. 1 to select the most active and productive isolates to complete the study.

The Congo Red method was used to measure the ability of bacteria to form biofilm as a qualitative method. Out of 50 isolates, 4 (8%) isolates showed their ability to produce biofilm. Isolates were classified as non-biofilm producers when the cutoff (OD= 0.120 - 0.240 or > 0.240) was strongly or moderately productive, as shown in Table 6.

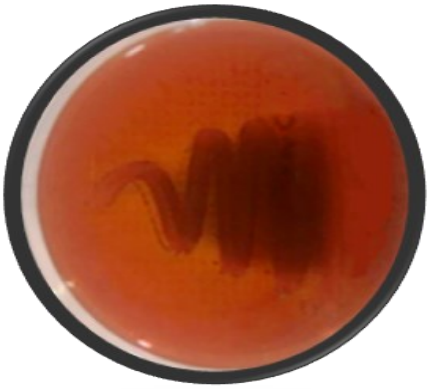
The result showed that producer100 % of isolates produced for PVD were biofilm producers (Table 6).The result showed that isolate PS3 was the weak producer of PVDand biofilm, while isolate PS1 and Ps2 were the strong producer, so PVD produced from it was selected to complete the study. Moreover, it showed that the more biofilm formation, the more PVD production, and vice versa (Table 6 and Fig. 2). The outcome demonstrated that every isolate that produced 100% of the isolates for PVD was a biofilm producer (Table 6). According to the results, isolated PS1 was the strong producer of PVD and biofilm, while isolated PS3 was the weak producer. Additionally, it showed that PVD production increased with biofilm growth and vice versa (Table 6 and Fig. 4).

All of the isolates used in the study were resistant to

Table 5. Phenotypic of antibiotic susceptibility of bacterial isolates in present study

Antibiotic disc	No. (%) of resistant isolate	No. (%) of intermediate isolate	No. (%) of sensitive isolate
Amikacin	35 (70%)	2(4%)	13 (26%)
Azteronam	50 (100%)	0(0%)	0(0%)
Ceftazidime	14 (28%)	15 (30%)	21(42%)
Ceftriaxone	24(84%)	0(0%)	8(16%)
Cepfepime	50 (100%)	0(0%)	0(0%)
Ciprofloxacin	24(48%)	17(34%)	9(18%)
Doripenem	19(38%)	0(0%)	31(62%)
Gentamicin	45 (90%)	1(2%)	4 (8%)
Imipenem	50 (100%)	0(0%)	0(0%)
Levofloxacin	21 (42%)	3(6%)	26 (52%)
Meropenem	21 (42%)	7(14%)	22 (44%)
Netilmicin	50 (100%)	0(0%)	0(0%)
Norfloxacin	20 (40%)	13 (26%)	17 (34%)
Ofloxacin	50 (100%)	0(0%)	0(0%)
Piperacillin	50 (100%)	0(0%)	0(0%)
piperacillin-tazobactam	13(26%)	8(16%)	29(58%)
Tobramycin	50 (100%)	0(0%)	0(0%)

A. *Biofilm formation in Ps1 isolate*



B. *Biofilm formation in Ps2 isolate*

Fig. 4 . *Biofilm formation assay on Congo red agar at 37 o C for 24 hr*

the 17 antibiotics used. Four of the isolates were selected formed biofilm, which is considered a virulence factor for *P. aeruginosa*, and these isolates produced pyoveridine (Table 5 and 6) .

After the exposure of the cells to different concentrations (400, 200, 100, 50, 25) to µg\ml from PVD of Ps1 and Ps2 isolates at a temperature of 37^{o}C , an inhibition was found in the cell growth at different rates based on the cell type and concentration with significant differences. PVD of Ps1 extract showed the highest inhibition affected compared with PVD of Ps2 and A549, which was affected more than A375.

Table 7 shows that the viability of A375 cell line was 24.267%, 10.967%, 10.767%, 7.867% and 7.667% when treated with 400µg\ml,200µg\ml,100µg\ml, 50µg\ml and 25µg\ml respectively for Ps1 PVD. F-test value (23.06) refers to significant differences as 400µg/ml showed significant differences from other concentrations (200, 100, 50, 25) µg/ml. 200µg\ml and 100µg\ml did not show any significant differences between them, but they differed significantly from 50µg\ml and 25µg\ml which have the same significant viability rate and the significant difference between the highest value (24.267% for 400µg\ml) and the lowest viability rate (7.667% for 25µg\ml) was 16.6 when (p≤0.01 From the table 7 and Fig. 5 the viability of A549 cell line was 34.500%, 22.833%, 10.933%, 7.733% and 7.400% when treating with 400µg\ml,200µg\ml,100µg\ml, 50µg\ml and 25µg\ml respectively from Methanol extract. The highest effect was at the concentration 400µg\ml .The viable cells were 34.500% while 50µg\ml showed the lowest significant value (7.733%) and the significant difference between them was 26.767 when (p≤0.01).

As shown in Table 8, Ps2 PVD extract reduced the cellular growth of both A549 and A375 cell lines. The highest percentage of inhibition was shown at 400 µg/ml on A549 cell line with a proportion of inhibition 55.60 % while the lowest percentage of inhibition was at 25µg/ml on A375 cell line with a proportion of inhibition 7.200% and the significant difference between them was 48.4 when (p≤0.01).

Table 8 and Fig.6 shows that the viability of A375 cell line was 31.800%, 18.300%, 9.233%, 8.100% and 7.200% when treated with 400µg\ml, 200µg\ml,100µg\ml, 50µg\ml and 25µg\ml respectively from PVD extract. PVD extract results for A375 cell line showed significant differences between them. 400µg/ml was significantly higher than other concentrations (200, 100, 50, and 25 µg/ml). 200µg/ml showed a significant difference from 100µg\ml, 50µg/ml and 25µg/ml also and there were no a significant differences between 100µg\ml, 50µg/ml and 25µg/ml when (p≤0.01).

Table 8 shows that the viability of A549 cell line was 55.600%, 26.433%, 14.767%, 9.700% and 8.533% when treating with 400µg\ml, 200µg\ml,100µg\ml, 50µg\ml and 25µg\ml respectively from Ps1 PVD. Results of PS2 PVD extract for A594 cell line showed significant differences between all concentrations except in 50µg/ml and 25µg/ml as there was no significant difference between them when (p≤0.01.)

Tables 7 and 8 showed that the best inhibition of cells was at 400µg/ml of Ps2 PVD extract and the A459 cell line was more affected with a proportion of inhibition 31.80%, while the 25 µg/ml from the same extract had the lowest effect with a proportion of inhibition 7.20% on A375 cell line and the significant difference between them was 24.6 when (p≤0.01).

Table 6. Quantitative determination of biofilm and pyoverdin production by 4MDR *Psuedomonas aeruginosa* isolates

P. aeruginosa	Biofilm production	PVD production
	OD570 nm	OD 405 nm
Ps1	0.524 ± 0.004	0.33 ± 0.004
Ps2	0.284 ± 0.004	0.14 ± 0.003
Ps3	0.132 ± 0.004	0.12 ± 0.004
Ps4	0.304 ± 0.004	0.18 ± 0.004

DISCUSSION

The positive result for *P. aeruginosa* on blood agar typically displays beta hemolysis and blue or green pigment

Table 7. Anticancer activity of *Ps1* pyoverdine extract on A549 and A375 cell lines by using MTT method after 24 h. of incubation at 37° C

Cell lines	Concentrations of extracts (µg/ml) (Mean ± stDev.)					F**- test
	400	200	100	50	25	
A 375	24.267± 2.80A	10.967±2.411B	10.767 ±1.795B	7.867± 3.325C	7.667± 1.818C	23.06
A549	34.500± 5.237A	28.833±3.302B	10.933±3.63Cd	7.733± 3.453D	7.400± 5.897C	18.82

** = (p ≤ 0.01); Similar letters mean the absence of significant differences

Table 8. Anticancer activity of Ps2 pyoverdine on A549 and A375 cell lines by using MTT method after 24 h. of incubation at 37°C

Cell lines	Concentrations of extracts (µg/ml) (Mean ± SDs.)					F**- test
	400	200	100	50	25	
A 375	31.800±9.789A	18.300±4.272B	9.233 ±1.294C	8.100± 2.902c	7.200± 1.706C	12.84
A549	55.600±4.328A	26.433±4.508B	14.767±6.279C	9.700± 3.365d	8.533± 3.251D	57.11

** = (p ≤ 0.01); Similar letters mean the absence of significant differences

(Kang and Kirienko, 2017). Cetrimide agar had a mucoid appearance, smooth in shape with flat sides and an elevated center, creamy in color, and smelled fruity. This is a selective medium for the isolation and presumed identification of *P. aeruginosa* that contained peptone, MgCl□, K□SO4, cetrimide and agar. It adds rehydrated contents of one vial of nalidixic acid selective supplement (FD130), which inhibited other microbial flora growing with *Pseudomonas* spp., and produced blue-green pigment (Kang and Kirienko, 2018).

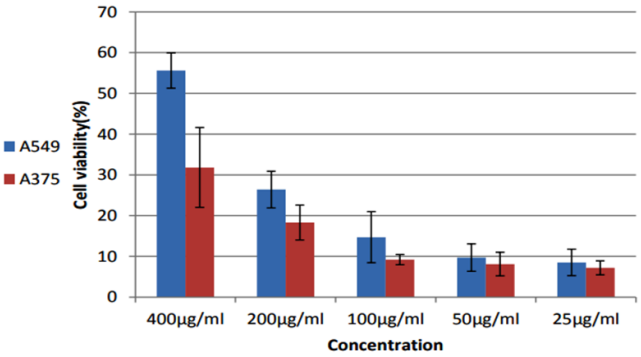


Fig. 5. *Anticancer activity of PVD extract of ps1on A549 and A375 cell lines by using MTT method after 24 h. and 37°C*

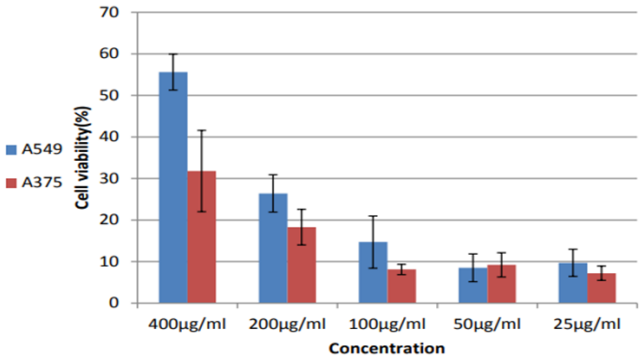


Fig. 6. *Anticancer activity of pyoverdine Ps2 on A549 and A375 cell lines by using MTT method after 24 h. 37° C.*

In biochemical testing, negative findings were obtained for the urease test, but good results were obtained for the oxidase and catalase tests. The isolates tested negative for indole, methyl red (MR), and Voges-Proskauer (VP) but positive for citrate utilization on Simmon's citrate agar, indicating that they could use citrate as their exclusive carbon source. Due to their rigorous aerobic nature and negative Gram's stain, the iron agar in Kligler has been given an alkaline slant without affecting the bottom and has remained Hydrogen sulfide H_2S negative without producing gas. The isolates were able to develop for 24 hours at 42˚C on cetrimide agar (Kang *et al*., 2020**)**

The present study revealed that *P. aeruginosa* was 100% resistant to piperacillin in 50/50 samples, while Kang *et al.* (2020) and Lima *et al*. (2021) reported resistant rates of 37.0%, 59.61% and 67.96%, this result is completely inconsistent. Although Madloom and Umran (2020) and Mahmoud *et al*. (2021) reported 100% and 85.4% resistance rates, respectively, the present result is comparable to or near those results. Beta lactam-beta-lactamase inhibitors combination antibiotics also showed resistance to piperacillin-tazobactam 13/50 (26%) . The resistance to cefepime was 50/50 (100%), close to the results of Othman *et al.*(2014), who reported a resistant rate of *P. aeruginosa* of ceftazidime, which was 100% .

The results of the present study are consistent with the results of Omidvari *et al*. (2021), as the isolates of *P. aeruginosa* showed high resistance to ceftriaxone, amikacin , piperacillin and were resistant to both ciprofloxacin (43%) and tobramycin (100%) and gentamicin (87%) and resistant to both ofloxacin and imipenem resistant (100%). The majority of isolates exhibited antibiotic resistance, particularly β- lactam antibiotics.

When iron is limited, bacteria produce siderophores, or iron-chelating chemicals, to take up iron from the host (Romling and Balsalobre, .2022)Pyoverdine, which

has a high affinity for Ps_2, and pyochelin, which has a low affinity, are the two types of siderophores that *P. aeruginosa* generates (Sasirekha and Srividya, 2016) . Another study examined the formation of pyoverdine pigment by 10 MDR *P. aeruginosa*. The findings indicated that 100% (10/10) of the bacteria were the producers of PVD siderophores (Sayyed *et al*.,2022) . Sebat *et al.* (2022) examined the development of PVD, a significant virulence factor, in 70 isolates of *P. aeruginosa* from children who had burns and wounds. Particularly in host tissues, PVD buildup was seen, and this accumulation was connected to host death.

Several studies have documented various bacteria (Vetrivel*et al*.,2021; Visaggio *et al* .,2015; Youzhou *et al*.,2012). *P. aeruginosa* FP6 produced an 85.7 lM siderophore in succinate medium (SM) (Aloush *et al.*,2016) It was determined from earlier research on the four MDR *P. aeruginosa* isolates' biofilm formation and PVD production that the greater biofilm formation, the more production, and vice versa.

In this regard, the outcomes of the Congo red agar biofilm formation experiment and the tissue culture plate approach demonstrated that 8% (4/50) of the isolates of *P. aeruginosa* developed biofilm, whereas 88% (46/50) did not (Minandri et al.,2016).

A quantitative method for assessing biofilm growth in a different study led to the classification of 77.5% (31/40) of *P. aeruginosa* isolates as biofilm producers. According to (Brandel *et al*.,2022), a phenotypic assay was considered the "gold standard" for biofilm recognition, with the following results: weakly adherent 42.5% (17/40), moderately adherent 27.5% (11/40), and firmly adherent 7.5% (3/40).

The TCP method was used for 10 MDR *P. aeruginosa* biofilms. The results indicated that 0% (0/10) of the biofilms were non-producers, 20% (2/10) were moderate producers, and 80% (8/10) were strong producers (Patel *et al.,*2003).

Cunrath et al. (2014) after screening 50 isolates came to the same conclusion that clinical *P.aeruginosa* isolates for biofilm development using the CRA and TCP. It was found that only six isolates generated biofilm, whereas 44 isolates were non-producers of biofilm.

Studies were conducted into the relationship between pathogenicity and biofilm production and the relationship between pyoverdine and biofilm production. According to investigations, pyoverdine synthesis requires biofilm generation (Roche *et al*., 2021).These studies also found a strong, statistically significant positive correlation between pyoverdine production and pathogenicity.

The present study supports the findings of Reimmann (2022), who reported obtaining 80 clinical isolates of *P. aeruginosa*. Twenty percent of multidrug-resistant *P. aeruginosa* isolates were examined to form biofilms and manufacture colors, and all three biofilm-related genes were detected and simultaneously in 87.5% (n = 70) of the bacteria. The isolates also demonstrated resistance to all medications.

A similar conclusion was reached by Braud *et al*. (2019) when they identified virulence factors, including biofilm formation, PVD production, and lasR gene, in 10 isolates of *P. aeruginosa* that were resistant to drugs. *P. aeruginosa* can create a strong biofilm structure by creating a biofilm matrix. Due to their diverse tolerance mechanisms, these bacterial biofilms are more naturally resistant to ordinary antibiotic therapy.The number of drug impairments associated with *P. aeruginosa* biofilm infections is increasing according to the current circumstances(Braud *et al.*,2019).

The lung cancer cells (A549) were more affected than the skin cancer cells (A375). This agrees with Reimann (2022), who demonstrated that crude extracts affect in different ways with cell lines. This may be due to the nature of the compounds found in each crude extract and their interaction with the metabolic nature of each type of cancer cell or to the effectiveness of some enzymes that act as antioxidants especially in cancer cells Saleh *et al.* (2015).

Conclusion

Pseudomonas aeruginosa is a hospital infection bacteria that contaminates wounds and burns and causes many infections. *P. aeruginosa* isolated from wounds and burns was resistant to multiple antibiotics and could produce biofilms. It was found that it could produce a pyoverdine dye that showed high anticancer effectiveness when tested against two lines of cancer using MTT assay. This result is of great importance in its use as an anticancer to contribute to the challenge of this dangerous disease, considered a problem of the times.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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