Iraqi Journal of Biotechnology, 2021, Vol. 20, No. 1, 63-69

Screening of Production Pyocin S5 Isolate from Multidrug Resistant *Pseudomonas aeruginosa*

Sura Saleem Albermani[1], Essam Fadel Alwan Al-Jumaili[2]

[1] Asst. Lec. Biology Dept. College of Science. Al-Farabi University Collage, Baghdad, Iraq.
[2] Professor, Biotechnology Dept. Genetic Engineering and Biotechnology Institute for Postgraduate Studies, University of Baghdad. Al-Jadriya Campus, 10071 Baghdad, Iraq.

Received: October 4, 2021 / Accepted: November 30, 2021 / Published: December 12, 2021

Abstract: This study included collecting (120) clinical specimen from different sources, (43) isolates successfully were diagnosed as *P. aeruginosa*, representing 35.8% of total isolates. The test for the antibiotics susceptibility of the bacteria to (10) types of antibiotics showed a clear variation in their resistance to antibiotics, as all isolates showed resistance (100%) against to cephalexin, (90 %) against to Gentamicin and 82% against to Ceftazidime,14 isolate out of 43 isolate had MDR. While the results of the phenotypic detection of pyocin production to resistance isolate (14 isolate) showed the presence of 12 isolates (85.7%), The third screening stage molecular detection of the pyocin S5 gene, which is one of responsible for the production of Pyocin S5, It was shown that 6 isolates (50%) possess this gene, while 6 isolates (50%), they did not possess this gene.

Keywords: *P. aeruginosa*, Bacteriocin, S5-type pyocin, Antibiotics, Production

Corresponding author: (Email: prof.dressamal-jumaily@igeuobaghdad.edu.iq).

Introduction

Pseudomonas aeruginosa part of normal intestinal flora and a powerful pathogen classified as an ESKAPE organism responsible for ICU-acquired infections in critically ill patients (1). That colonizes a diverse range of habitats water, plants, soil, on the epidermis of animals including and surgical equipment and catheters. In nature, it is commonly found as a plankton swimming through water (2, 3). Bacteriocins are a large family of ribosomally synthesized functionally and environmentally diverse proteinaceous toxins produced by archaea, produced and bacteria for intra- and inter-species competition (4). Which are produced as a secondary metabolite by many bacteria and have the ability to oxidize and reduce other molecules, exhibiting killing activity or growth inhibition (5). Being an inhabitant of all environments, from aquatic to terrestrial, from soil to distilled water, from plants to humans, *P. aeruginosa* is the quintessence of microbial arms depot. It produces a wide range of secondary metabolites to protect its niche from other fungi and distantly related bacteria. In order to fight fellow *Pseudomonads* and other closely related bacterial species that may compete for common niches, all strains of *P. aeruginosa* also produce a broad range of bacteriocins referred to as pyocins. The two major groups of pyocins produced by *P. aeruginosa* are (i) S-type pyocins (colicin-like bacteriocins), (ii) Tailocins (high-molecular weight bacteriocins that resemble phage tails). The structure of S-type pyocin is similar to that of colicin except that many S-type pyocins have three domains (6); domain I is N-terminal that recognises the cell surface receptor, domain II has unknown

function and domain III translocate and penetrates pyocin, C terminal domain carries out the killing activity (7,8). S-type pyocin are rather small proteins similar to colicin (bacteriocin produced by *Escherichia coli*), water-soluble and protease and heat-sensitive. These bacteriocins are secreted as binary protein complexes consisting of a protein with killing activity and has two protein components; one is a larger than the other (7). The larger component carries out the killing activity called effector component (9) and the smaller component is an immunity protein that is like the one in colicin, this protein protects the host cell from the killing activity of the larger component. In this study we detect the ability of local *P. aeruginosa* isolates to produce pyocin and detect the genes that coding of S5-pyocin in these isolates.

Materials and Methods

Collection and Identification of Bacterial isolates

One hundred and twenty clinical specimen were collected from several hospitals in Baghdad city (Children Welfare Teaching Hospital Educational Laboratories and Burns Specialist Center) belong to Medical City, Mohammed Baqer Al-Hakim Hospital during period from the January 2020 to the July of 2020 from various clinical sources (urine, blood, ear and burin). *P. aeruginosa* isolates identified according to morphological, microscopic, biochemical tests according to (10, 11) and the VITEK® 2 Compact system (BioMe'rieux, French) is dedicated to the identification of clinically significant bacteria.

Antibiotics Susceptibility Test

The susceptibilities of the isolates to 9 antibiotics (Himedia, India) by Kirby-Bauer disk diffusion method (12): ceftazidime 30 µg, gentamycin 10 µg, tobramycin 10 µg, imepenem 10 µg, ciprofloxacin 5 µg, lomefloxacin 10 µg, norfloxacin 10 µg, levofloxacin 5 µg and amikacin 30µg, were determined on Mueller-Hinton agar by the Kirby Bauer disk diffusion method. The zone of inhibition diameter was measured and the results were interpreted based on t1`1`he guidelines by the Clinical and Laboratory Standards' institute (13).

Production of crude pyocins

Production of pyocin was augmented by manipulating a variety of physical and chemical parameters. Extracellular pyocin was obtained by incubated overnight single colony of *p.aeruginosa* in glutamate (G) medium was used for the induction of pyocin production (20 g sodium glutamate, 5 g glucose, 0.1 g MgSO4·7H2O, 5.63 g Na2HPO4·12H2O, 0.25 g KH2P04, 0.5 g yeast extract, and 50 mg tryptophan dissolved in liter of distil water), 10 g (1g:100ml) $KNO_3$was added to the culture (1 liter) and incubation was carried out at 37 °C. The culture was then shaken with 10 mL of chloroform and cold centrifuged at 8000 g for 20 min to remove the cell debris. Control cultures containing no KNO_3 were also investigated (14).

Agar disk diffusion

For detect strain product to pyocin In *vitro*, by inhibitory activity against different strains of bacteria by the agar-disk diffusion assay (15). An aliquot of

20 µl of partially purified antimicrobial substance was applied to disks (6 mm) placed on agar plates previously inoculated with a swab submerged in an indicator strain A 24-h nutrient broth culture at 37 °C of the pyocins-sensitive strain suspension which corresponded to a 0.5 McFarland turbidity standard solution.

Detection of PyoS5 genes

Genomic DNA was isolated from bacterial growth according to the protocol of ABIOpure, while electrophoresis was carried out according to (16). The polymerase chain reaction was conducted in laboratory conditions. BAGEL 3 and 4 (BActeriocin GEnome mining tool were used to identify in-silico genes encoding S5-type pyocins (17). Primer used in this study was designed by using the *P. aeruginosa* Genome Database as a reference. These primers were supplied by Macrogen Company in a lyophilized form. The PyoS5 gene with a Amplicon size of 769 bp and its genetic sequence (PYOF_ACTGCCATTACCAGTGCG AA,PYOR_TTGATCAAGCGGGAAG TGCT) which was designed for this study, while the interaction program for each gene was approved as shown in Table (1).

Table (1): PCR Program of pyos5 gene.

Steps	°C	m: s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	55	00:30	
Extension	72	00:30	
Final extension	72	07:00	
Hold	4	10:00	1

Results and Discussion

Isolation of *P. aeruginosa*

Out of 120 specimens that were collected from the five hospitals, 43 isolates successfully were diagnosed as *P. aeruginosa*, representing 35.8% of total specimens and the highest percentage of *P. aeruginosa* was obtained from burn samples 21 isolates (%48.8) whereas the lowest percentage were obtained from otitis samples 3 isolates (%6.9). *P. aeruginosa* is the third most prevalent pathogen related with hospital-acquired infections, according to (18), who found the greatest percentage of *P. aeruginosa* among burn infections, followed by wound and ear infection (19) and expected with high prevalence of *P. aeruginosa* in community may be related to the increasing numbers of the immune compromised patients in our population due to different diseases and contaminations of the environment in hospital and in special patients with long stay in hospital. This agrees with several studies conducted by (20, 21).

Identification of *P. aeruginosa*

In the laboratory, *P. aeruginosa* is able to grow on a wide variety of non-selective agar including nutrient agar and broth, blood agar and MacConkey agar, ranging from minimal to complex. On MacConkey agar medium the colonies of *P. aeruginosa* isolates appeared 2-3 mm, flat, smooth, non-lactose fermenting colonies these colonies have regular margins. B-haemolysis is observed on Blood Agar represented by the clear zone around the

colonies. Blood agar *P.aeruginosa* produces mucoid-type colonies with a typical metallic sheen. Cetrimide agar medium *P. aeruginos*a colonies (greenish-blue in color) are medium-sized and characterized by an irregular growth. The visual examination of the plates is performed by using ultraviolet light to detect the presence of fluorescein (22,23,24).

Identification of *P. aeruginosa* by Vitek 2 system

The final identification was performed with the automated Vitek2 system using GN-ID cards which contained (64) biochemical tests. The results demonstrate that all (43) isolates for *P. aeruginosa* were confirmed with ID message confidence level ranging excellent (the probability percentage was 99%-95%). This approach is distinguished by its ability to identify bacteria quickly without the need of a large number of culture media, as well as its ability to decrease culture contamination. It is a new tool for the rapid identification of Gram-negative bacteria from human clinical specimens (25).

First screening for resistance antibiotic bacteria

Results of antibiotics susceptibility test were exhibited that total of the 43 isolates obtained in this study a clear variation in their resistance to antibiotics, with highest resistance percentage (100%) observed against to cephalexin, (90 %) against to Gentamicin and 82% against to Ceftazidime. Also, results revealed a varying degrees of susceptibility to tested antibiotics, high percentage of *P. aeruginosa* isolates were sensitive to Aztreonam and Amikacin in the percentages (63.4%), (55.5%), respectively. Ciprofloxacin (47.5%), Imipenem (20.2%), Levofloxacin (38.4%), Tobramycin (27%) and Ofloxacin (18.3%) were revealed intermediate action against the isolates.

Several studies have demonstrated a relationship between pyocin and multiple antibiotic resistance, as pyocin induces alterations in LPS. This hinders the permeability of the outer membrane to antibiotics. (26) Isolates of *p.aeruginosa* antibodies resistant that has the highest productivity of pyocin. The bacterial resistance to ciprofloxacin by regulating pyocin biosynthesis genes in *P. aeruginosa* (27,28). For this, the resistant isolates were selected to test pyocin productivity.

Second screening phenotypic detection of pyocinogeny bacteria

Exposing *P. aeruginosa* strains to stress factors was effective in increasing expression of bacteriocins. The 14 resistant isolates selected to test pyocin productivity, Potassium Nitrate (KNO_3) treatment was found to be effective in inducing pyocin S5 production. The turbidity of pyocinogenic cell culture continued to increase for about 1 h after the addition of the KNo_3 to the culture at the logarithmic phase, and then rapid lysis ensued with the concomitant release of pyocin S activity twelve isolate (12 or 85.7%) figure (1), resistant *P. aeruginosa* exhibited pyocin activity against the *S. aureus* were previously isolated and reported was selected for further study. The inhibitory diameters ranged between (11-30 mm) (29). Such bio-active potential might be attributed to the presence of high number of bacteriocin adsorption receptors in the peptidoglycan-based cell wall of Gram-positive bacteria (30).

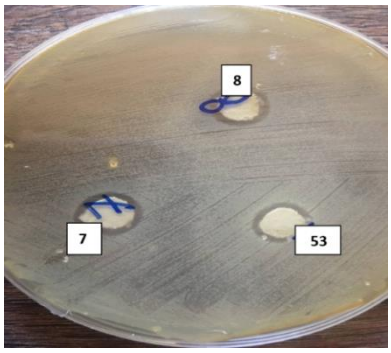

Figure (1): Antibacterial activity of the pyocin crude on sensitive bacteria indicator.

Third screening detection of PyoS5 genes in pyocinogeny bacteria

Extraction of DNA

The DNA of 12 bacterial isolates of *P.aeruginosa* (obtained from growing on cetrimide agar medium) was extracted and purified by using genomic DNA purification kit. The results were detected by electrophoresis on 1% agarose and exposed to U.V. light in which the DNA appears as compact bands.

Detection of pyocin S5 genes

All the isolates which were positive for pyocin production were subjected to specific primers pyocin S5. Results were showed that 6/12 (50%) isolates were positive to pyocin S5 gene. The DNA of isolates were amplified by PCR technique to detect pyocin S5 gene. The results of PCR amplification were confirmed by electrophoresis analysis. By this analysis the strands of DNA which resulted from successful binding between specific pyoS5 primer and the extracted DNA template appear as single band under U.V. light using ethidium bromide as a specific DNA stain figure (2).

The pyocin S5 gene have amplicons size (769 pb), were estimated depending on DNA marker (100 bp DNA ladder). The results are differing with (31), they are found pyocin S5 gene 22%. Another study search on other pyocin gene (1S,2S,3S), previously same the result of (32) considered as a local study where the study illustrated that prevalent rate of appearance S1S2 gene is (48%) while different from (33) appearance S1S2 gene is (90%) while prevalent rate of appearance S3gene is (82%).

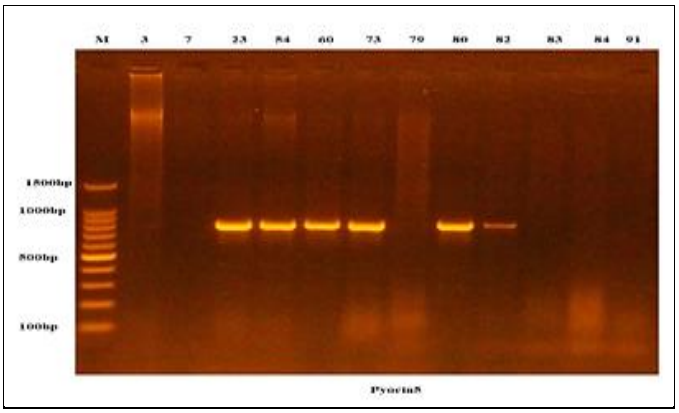

Figure (2): Amplification results of pyocin S5 primers in *P. aeruginosa* samples fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-12.

Conclusions

Our study revealed the efficacy of Aztreonam and Amikacin in inhibiting *P.aeruginosa*, 85.7% from resistant isolates had ability to produce pyocin (R,F,S) detection by inhibition zone (phenotype method), pyocin S5 were found in 50% from resistant isolates.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; and agreed to be accountable for all aspects of the work.

References

1. Pachoria, P.; Gothalwalb, R. and Gandhi, P. (2019). Emergence of antibiotic resistance *Pseudomonas aeruginosa* in intensive careunit; a critical review. Genes and Diseases, 17;6(2):109-119.
2. Streeter, K. and Katouli, M. (2016). *Pseudomonas aeruginosa*: a review of their pathogenesis and prevalence in clinical settings and the environment. Infectious Epidemiology and Microbiology, 2(1): 25-32.
3. Otero-Asman, J. R.; García-García, A. I.; Civantos, C.; Quesada, J. M. and lamas, M. A.(2019). *Pseudomonas aeruginosa* possesses three distinct systems for sensing and using the host molecule haem. Environmetal Microbiology, 21(12):4629-4647.
4. West, S. A.; Diggle,S. P. A. Buckling, A.; Gardner, A. and Griffins, A. S.(2007). The social lives of microbes. Annual Review of Ecology, Evolution, and Systematics (1)38:53–77.
5. Mehnaz, S. (2017). Rhizotrophs: Plant Growth Promotion to Bioremediation. Microorganisms for Sustainability. 2:978-981.
6. Elfarash, A.;Dingemans, J.; Ye, L.; Hassan, A. A., Craggs, M.; Reimmann C, Thomas; M.S. and Cornelis, P. (2014). Pore-forming pyocin S5 utilizes the FptA ferripyochelin receptor to kill *Pseudomonas aeruginosa*. Microbiology, 160(2):261-269.
7. Michel-Briand, Y. and Baysse, C. (2002). The pyocins of *Pseudomonas aeruginosa*, Biochimie, 84(5-6):499-510.
8. Duport, C.; Baysse, C. and Michel-Briand,Y. (1995). Molecular characterization of pyocin S3, a novel S-type pyocin from *Pseudomonas aeruginosa* . Journal of Biological Chemistry., 270(15):8920-7.
9. Lee, F. K. Dudas KC, Hanson JA et al. (1999) The R-type pyocin of Pseudomonas aeruginosaC is a bacteriophage tail-like particle that contains a single-stranded DNA. Infectious Immunology. 67(2): 717–725.
10. MacFaddin, J. E. (2000). Individual biochemical tests for identification of medical bacteria.3th ed. Lippincott Williams Wilkins. London. 912.
11. Mahon, C.;Lehman, D. and Manuselis G. (2007) Textbook of Diagnostic Microbiology, 3rd Edition, Elsevier. P. 508.
12. Harley, J. P. (2016). Laboratory Exercises in Microbiology. 10th ed. McGraw-Hill Higher Education. New York.
13. CLSI. (2020). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.
14. Ohkawa, I.; Kageyama, M. and Egami, F. (1973). Purification and properties of pyocin S2. Journal of Biochemistry, 73(2):281-9.
15. Motta, A. S. and Brandelli, A. (2002). Characterization of an antibacterial peptide produced by Brevibacterium linens. Journal of Applied Microbiology, 92(1):63-70.
16. Sambrook, J. and Rusell, D. W. (2001). Molecular cloning: a laboratory manual. Cold spring Harbor , NY : Cold spring Harbor laboratory press.
17. van Heel, A. J.; de Jong, A.;Montalban-Lopez, M.; Kok, J. and Kuipers, O. P. (2013). BAGEL3: automated identification

of genes encoding bacteriocins and (non) bactericidal post translationally modified peptides. Nucleic Acids Research 41,448–53.

18. R'auf, W. M. (2003). Bacteriological and genetical study on disinfectants exposed *Pseudomonas aeruginosa* [Ph.D. Thesis]. The College of Medicine, University of Takrit.
19. Moreau-Marquis, S.; Stanton, B. A. and O'Toole, G. A. (2008). *Pseudomonas aeruginosa* biofilm formation in the cystic fibrosis airway. Pulm. Pharmacology. Therapy. 21(4): 595–599.
20. Saleh, M. M.; Sadeq, R. A.; Latif, H. K. A.; Abbas H. A. and Askoura M. (2019). Zinc oxide nanoparticles inhibits quorum sensing and virulence in *Pseudomonas aeruginosa*. Africane Health Science. 19(2):2043-2055.
21. Pang, Z. ;Raudonis, R.;Glick, R. B.;Lin, T.;Cheng, Z. (2019). Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. Biotechnology Advances, 37(1):177-192.
22. Al-Sheikhly, M. A.; Musleh, L. N. and AlMathkhury, H. J. (2020). Gene Expression of pelA and pslA in *Pseudomonas aeruginosa* under Gentamicin Stress. Iraqi Journal of Science, 61(2): 295-305.
23. AL-Fridawy, R. A K.; Al-Daraghi, W. A. H. and Alkhafaji, Marwa H. (2020). "Isolation and Identification of Multidrug Resistance Among Clinical and Environmental *Pseudomonas aeruginosa* Isolates." Iraqi Journal of Biotechnology. 19 (2): 1-8.
24. Al-Daraghi, W. A. H. and Al-Badrwi, M. S. A. (2020). Molecular Detection for Nosocomial *Pseudomonas aeruginosa* and its Relationship with multidrug Resistance, Isolated from Hospitals Environment .Medico-legal Update, 20(1), 631-636.
25. Feng, W.;Sun, F. j.;Wang, Q.;Xiong, W.;Qiu, X. ;Dai, X. and Xia P. (2017). Epidemiology and resistance characteristics of *Pseudomonas aeruginosa* isolates from the respiratory department of a hospital in China. Journal of Global Antimicrobial Resistance, 8,142-147.
26. Saeed, A. Y.; Ahmed, D. F.; Saleh, M. K.; Alazzawie, A. F.; Mostafa, M. Q., Mahdii, F. M., Shehab, N. W. and Al Hashimi, O. A. M. (2021). Phenotypic and Molecular Detection of Pyocin from Multidrug Resistant *Pseudomonas aeruginosa* Isolated from Various Pathogenic. Indian Journal of Forensic Medicine & Toxicology, 15(2)1659-1667.
27. Long, Y.; Fu, W.; Wang, S.; Deng, X.; Jin, Y.;Bai, F.; Cheng, Z. and Wu, W. (2020). Fis Contributes to Resistance of *Pseudomonas aeruginosa* to Ciprofloxacin by Regulating Pyocin Synthesis. Journal of Bacteriology, 202(11), 64-20.
28. Chen, F.; Chen, G.; Liu, Y.; Jin, Y.; Cheng, Z.; Liu, Y.; Yang, L.; Jin. S. and Wu, W. (2017). *Pseudomonas aeruginosa* oligoribonuclease contributes to tolerance to ciprofloxacin by regulating pyocin biosynthesis. Antimicrobial Agents Chemother,apy 61(3): 2256-16.
29. Heo, Y. J.; Kwan, S. K.; Song, J. H. and Cho, Y. H. (2005). Competitive growth advantages of various *Pseudomonas aeruginosa* strains. Journal of Microbiology and Biotechnology., 15(6): 1368-1376.
30. Padilla, C.;Lobos, O.; Brevis, P. and et al. (2002). Effects of the bacteriocin PsVP-10 produced by *Pseudomonas* sp. on sensitive bacterial strains. De Microbiologia, 44(1):19-23.
31. Bara, J. J.;Matson, Z. and Remold, S. K. (2018). Life in the cystic fibrosis upper respiratory tract influences competitive ability of the opportunistic pathogen *Pseudomonas aeruginosa*. Romanian Society open Science, 5: 180623.
32. Essa, R. H.; AL-Tamimi, H. D. O. and Rasool, K. H. (2016) Detection Genes Encoding Pyocin Production of *Pseudomonas aeruginosa*. World Journal of Pharmaceutical Science, 4(11):119-125.
33. Al-Shammary, A. H. R.; Auda, I. G. h. and Aziz, I. H. (2013). Pyocin-Based Molecular Typing of Local Isolates of *Pseudomonas aeruginosa* Isolated from Blood Samples. Iraqi Journal of Medical Science, 11(1).1-13.