Microbes and Infectious Diseases 2021; 2(4): 790-796

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Effect of *Lactobacillus plantarum* on virulence factors of *Pseudomonas aeruginosa* isolated from wound infection

***Maii Atef Shams Eldeen* [*1], *Ayat Shaban Elnahal* [2], *Mohamed Ibrahim ElSawaf* [3], *Abd-ElRehem Ghareeb Ads* [1], *Sara Mina Samy* [1]**

1. *Medical Microbiology & Immunology department, Faculty of Medicine-Tanta University- Egypt.*
2. *Medical Microbiology & Immunology department, Faculty of Medicine - Kafr-Elsheikh University- Egypt.*
3. *General Surgery department, Faculty of Medicine-Tanta University- Egypt.*

ARTICLE INFO

Article history:
Received 4 August 2021
Received in revised form 11 September 2021
Accepted 12 September 2021

Keywords:
Pseudomonas aeruginosa
Virulence
Probiotic
Lactobacillus plantarum

ABSTRACT

Background: *Pseudomonas aeruginosa* represents a major concern in nosocomial infections. It possesses many virulence factors that aid its pathogenicity and antimicrobial resistance. Non-antibiotic therapy against this pathogen includes probiotics, phages, phytomedicine and other new modalities. These agents alone or in combination with antibiotics can be highly effective against these virulent strains. **Objectives**: To investigate the in-vitro effect exerted by the probiotic "*Lactobacillus plantarum"* on two virulence factors of *Pseudomonas aeruginosa* (pyocyanin pigment production and elastase enzyme activity). **Methods:** The study was carried out on 40 *Pseudomonas aeruginosa* strains isolated from pus collected from wound infection using sterile swabs. The effect of *Lactobacillus plantarum* on pyocyanin production and the elastolytic activity of *Pseudomonas aeruginosa* were detected using pyocyanin extraction method and Human Elastase ELISA Kit respectively before and after the addition of *Lactobacillus plantarum*. **Results**: Among the forty strains investigated, the pyocyanin production and elastolytic activity of *Pseudomonas aeruginosa* isolates were significantly decreased after addition of *Lactobacillus plantarum.* **Conclusions:** *Lactobacillus plantarum* has an important invitro anti-pathogenic effect against *Pseudomonas aeruginosa* as it significantly interferes with two of its main virulence factors (pyocyanin & elastase).

Introduction

Pseudomonas aeruginosa (P. aeruginosa) is an ubiquitous pathogen that represents a significant problem in all health-care systems especially those requiring prolonged hospitalization [1]. The presence of such pathogen in medical micro-environments e.g. wounds exhibits major concerns in patients' post-operative care [2].

There are several virulence contributing factors of *P. aeruginosa* including cell-related factors e.g (endotoxin, flagella, pili) and extra-cellular ones e.g (alginate, exotoxin A, exoenzyme S, pyocyanin, elastase, etc.) [3].

Pyocyanin is a bluish cytotoxic pigmentation that acts by blocking the wound

DOI: 10.21608/MID.2021.92514.1185
* *Corresponding author:* Maii Atef Shams Eldeen
E-mail address: mai.eldeen@med.tanta.edu.eg

healing process through promoting the oxidative-stress process and activating the pathway of P38 mitogen-activated-protein-kinase (MAPK) in the infected tissues [4].

Pseudomonas is resistant to many antimicrobial agents either naturally or through acquired pathways and it becomes increasingly resistant to many discovered anti-Pseudomonal agents. Therefore, treatment of wounds or burn patients infected with *P. aeruginosa* became very problematic due to limited therapeutic alternatives [5] making it necessary to advance the search for other new non-antibiotic alternatives that are economically available to the developing countries [6].

Non-antibiotic therapeutic modalities against this multi-drug resistant (MDR) pathogen include probiotics, phages and phytomedicines. These agents by themselves or combined with currently used antimicrobials can be highly efficient against MDR *P. aeruginosa* strains [7].

Probiotics are viable non-pathogenic organisms capable of altering the flora of the medium where they exist e.g. gastrointestinal tract (GIT). Generally, they obtain a positive impact on the host health and physiology of the body [8]. They have also shown antiviral, anti-mycobacterial and anti-cancer effects [9].

Lactic acid bacteria (LAB) are a famous example of probiotics widely distributed in nature. Many studies designated their several health promoting and immunity function influences like regulation of gut microbiota disorders, stimulation of IgA production, enhancing T-cell production and providing a shield against other enteric organisms through inhibiting their growth, secreting acidic metabolites, bacteriocins and other products with antimicrobial effects [10-13].

Lactobacillus plantarum (*L. plantarum)* possesses anti-pathogenic ability against various pathogens e.g. *Escherichia coli, Shigella and Salmonella* [14]. It also shows immune-modulatory action, tissue repair, and angio-genic properties; suggesting that its use in the treatment of infected wounds is reasonable [15]. In this study we aimed to investigate the invitro effect of *L. plantarum* on two of the main extracellular virulence determinants of *P. aeruginosa* (pyocyanin pigment production and elastase enzyme activity).

Methods

Study population, setting, and data collection

The present study was carried out on forty pathogenic *P. aeruginosa* strains isolated from wound pus collected from patients admitted to Surgical and Burn Units of Tanta University Hospitals using sterile cotton swabs. The laboratory identification and procedures were conducted in Medical Microbiology and Immunology Department, Faculty of Medicine, Tanta University. Ethical approval for this study (no. 31186/11/2016) was provided by the Ethics and Research Committee, Tanta Faculty of Medicine. Written informed consents were obtained from all participants in this research. A code number was put to each sample for adequate provision to maintain privacy of participants and confidentiality of data.

Subjects

The study included clinically suspected patients who showed evidence of local signs for wound infection (Redness, hotness, swelling, purulent discharge or delayed healing of wound) and/or systemic manifestations (fever, chills, or hypotension) with no other apparent source of infection except the wound. Patients showing any other apparent or suspected source of infection were excluded.

Specimen collection

All samples were collected with sterile swabs in sterile containers under complete aseptic conditions. Swabs were introduced into the depth of lesion and rolled to aspirate pus or exudation from the wound. Samples were transported as soon as possible to Medical Microbiology and Immunology Department, Faculty of Medicine, Tanta University.

Specimen processing

Swabs were cultured on nutrient agar and MacConkey agar (Oxoid, UK) for evaluation of the colony size, shape, edge, color, opacity, elevation and surface. The organisms showing characteristic colony morphology of *P. aeruginosa* were obtained followed by microscopic identification by Gram stained films to clarify the morphology of the bacterial cells (size, shape and arrangement). *P. aeruginosa* was identified as Gram-negative bacilli of variable size, non-sporing and non-capsulated. Biochemical identification was performed with a simplified scheme of biochemical tests such as sugar fermentation test, triple sugar iron test (to confirm its non-fermentation activity) and oxidase test [16].

Preparation of *Lactobacillus plantarum* strain

A control strain of *L. plantarum* was kindly supplemented by Microbiology Department, Faculty of Pharmacy, Tanta University. It was

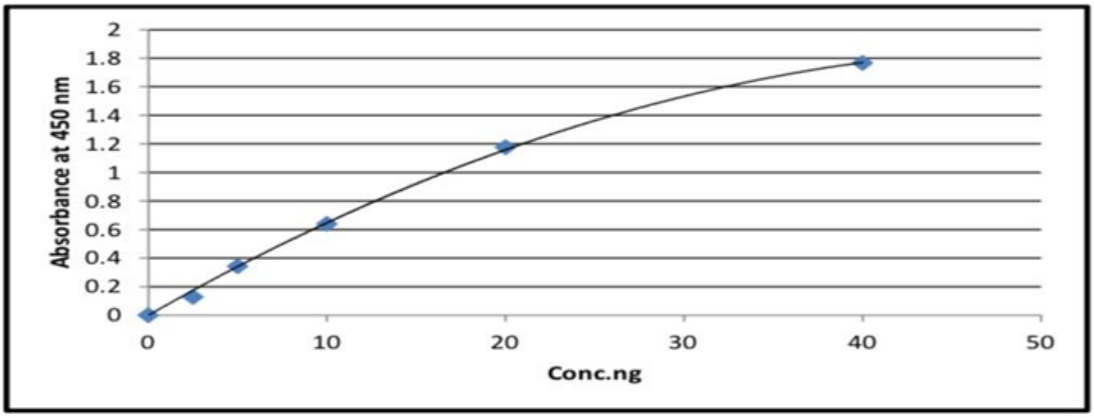
grown on blood agar at 37°C in a candle jar overnight. The *L. plantarum* colonies were identified as very minute alpha hemolytic colonies, Gram positive bacilli and catalase test negative [16].

- **Pyocyanin extraction**: The compound was isolated from cellular debris in an aqueous suspension formed by the addition of chloroform. Because of the unique pigmentation this compound was quantified with a spectrophotometer before and after addition of *L. plantarum*. All isolated *P. aeruginosa* strains which had the ability of pyocyanin pigment production were grown in presence and absence of *L. plantarum* for detection of ability of *L. plantarum* to interfere with pyocyanin pigment production before and after its addition [17].
- **Elastase assay***:* The elastolytic activity of the isolated strains were investigated by measuring elastase levels in all samples, before and after addition of *L. plantarum* using Elastase ELISA kit (Assay Kit Co., LTD) which is based on a standard sandwich enzyme-linked immune-sorbent assay technology [18]. Steps were followed according to manufacturers' instructions and O.D. absorbance was measured at 450 nm by an ELISA reader as shown in **table (1)** and **figure (1)**. A standard curve was drawn and elastase concentration in the samples was determined before and after addition of *L. plantarum*.

Table 1. Typical standards of the Elastase ELISA Kit**.**

Conc. ng/ml	**2.5**	**5**	**10**	**20**	**40**
Absorbance 450 nm	0.128	0.343	0.638	1.176	1.767

Figure 1. Standard curve Elastase ELISA Kit.



Statistical analysis

The raw data were coded, entered and analyzed using SPSS system files (SPSS package version 18).

Results

Pyocyanin production of *P. aeruginosa* isolates before and after addition of *L. plantarum*

out of the 40 *P. aeruginosa* isolates only 20 isolates showed pyocyanin production). The pyocyanin production in *P. aeruginosa* isolates was significantly decreased after addition of *L. plantarum* compared to pyocyanin production before addition of *L. plantarum* (*p.* value < 0.01*). This is shown in **table (2)** and **figure (2).**

Elastolytic activity of *P. aeruginosa* isolates before and after addition of *L. plantarum*

The elastolytic activity of *P. aeruginosa* isolates significantly decreased after addition of *L. plantarum* compared to elastase production before addition of *L. plantarum* (*p* value <0. 01*). This is shown in **table (3)** and **figure (3)**.

Table 2. Comparison between pyocyanin production of *P. aeruginosa* isolates before and after addition of *L. plantarum*.

Effect of pyocyanin production	Before	After	*p*. value
Mean ± SD	31.9 ± 13.19	23.11±8.07	<0.01*

Figure 2. Comparison between pyocyanin production of *P. aeruginosa* isolates before and after addition of *L. plantarum.*

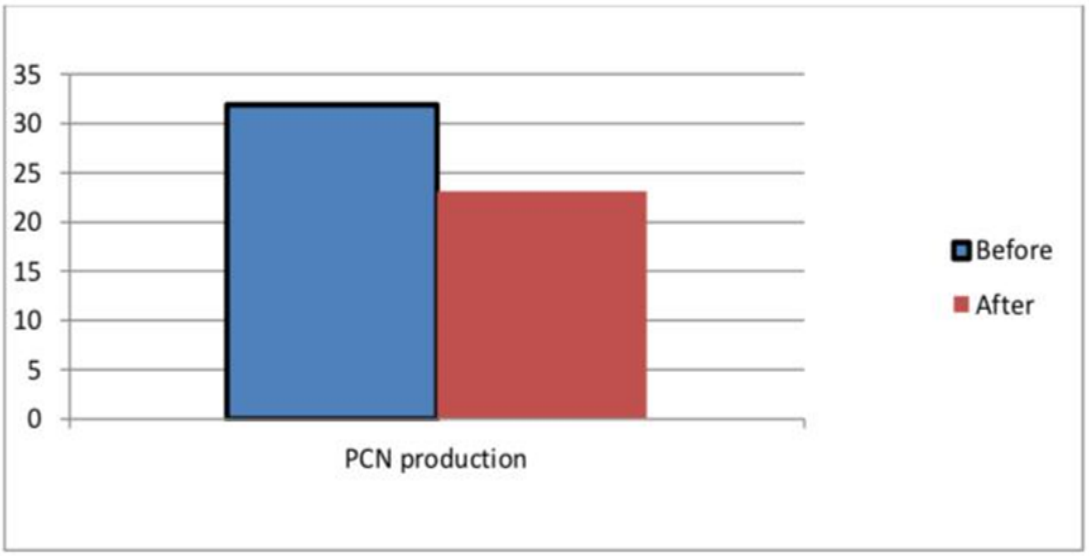
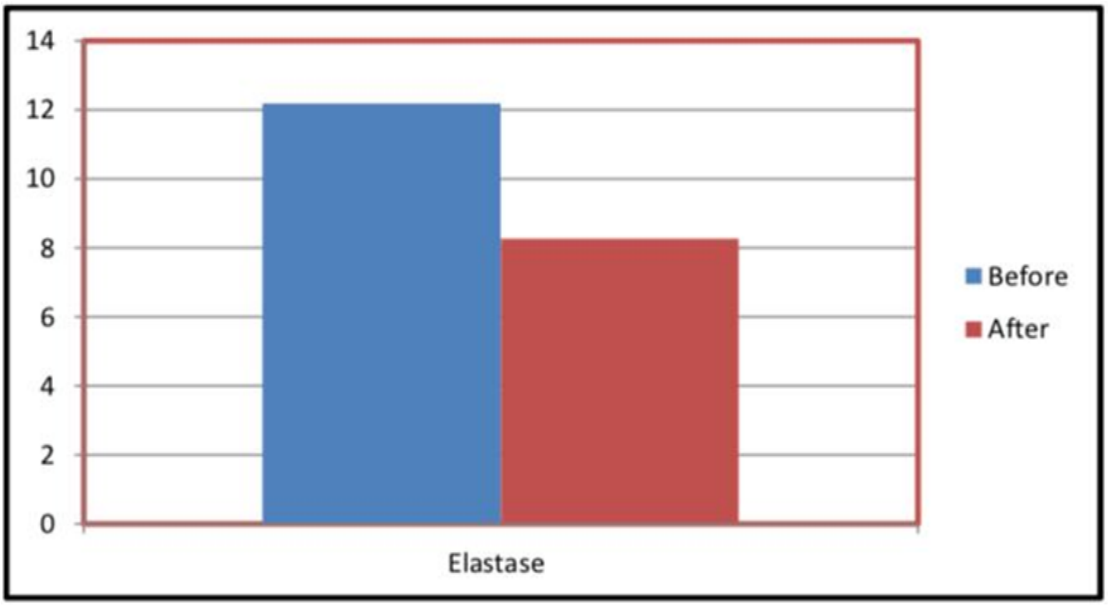


Table 3. Comparison between elastolytic activity of *P. aeruginosa* isolates before and after addition of *L. plantarum.*

Effect on elastase activity	Before	After	*p* value
Mean ± SD	12.18 ± 9.14	8.27 ± 6.43	0.007 (<0.01*)

Figure 3. Comparison between elastolytic activities of *P. aeruginosa* isolates before and after addition of *L. plantarum*.



Discussion

Multidrug resistant infections caused by virulent *P. aeruginosa* strains are increasing worldwide and the urgent need for novel yet nontoxic available antimicrobial agent is clear and mandatory**.**

This study investigated the invitro effect of the probiotic, *L. plantarum*, on two important *P. aeruginosa* virulence factors; we revealed that both pyocyanin production and the elastolytic activity in the examined *P. aeruginosa* isolates significantly decreased after addition of *L. plantarum*. Our results are concurrent with results of an Argentinean study [15] which also reported that both pyocyanin production and elastase enzyme concentration was significantly inhibited by *L. plantarum* suggesting that this significant inhibition of these two vital virulence factors was attributed to interference with the quorum-sensing (QS) mechanism which regulates the production of both pyocyanin and elastase enzyme as well as having a significant role in bacterial growth. Many recent studies have also confirmed *L. plantarum* role in controlling QS process exerting an inhibitory function against virulent strains of *P. Aeruginosa* and other related species e.g. Aeromonas [19-21].

For further clarification, another Egyptian study [22] reported that *L. plantarum* showed the capacity not only to inhibit the bacterial growth *P. aeruginosa* but also to interfere with the biological activity of acyl-homoserine lactone hormone responsible of the QS process [23] making it logical to observe an inhibition in the production of pyocyanin and elastase enzyme.

Moreover, our study is in partial agreement with another study [24] which reported significant inhibition of the elastolytic activity of *P. aeruginosa* after addition of *L. plantarum*. The inhibitory effect shown was associated with the inhibition of QS molecules, but an inhibitory action of *L. plantarum* secondary metabolites on elastase couldn't be ruled out.

On the other side, a French study [13] reported that when more than eighty *Lactobacilli* isolates were tested for their capacity to inhibit the elastolytic activity of *P. aeruginosa* control strain (PAO1), only five *Lactobacilli* strains could significantly reduce the activity of elastase. The discrepancy in results may be explained by the different *Lactobacilli* strains used and also the authors of the previous study explained that PAO1 is sensitive to pH and acid in a dose-dependent manner, growth inhibition increase in parallel with any increase in acid concentration and pH decrease. For that reason, the inhibitory activities of *Lactobacilli* towards *P. aeruginosa* elastolytic activity were noticed in those five strains in particular as they could induce a much higher pH decrease while other strains were poorly acidifying.

Conclusion

This study investigated the effect of *L. plantarum*, one of the probiotics, on pathogenic *P. aeruginosa* which became a major cause of nosocomial infections facing healthcare systems. Probiotics are thought to be a safe and economic alternative to antimicrobial agents that became highly resistant and ineffective against many pathogens during the last decades. Based on our results we can conclude that the in-vitro activity of two of the main virulence factors *P. aeruginosa's* was significantly diminished after addition of *L. plantarum.* It is possible that in near future it can be used as an in-vivo alternative medicine for the treatment of *P. aeruginosa* infections. However, **t**here were some limitations of this study. Firstly, the study could have been generalized, if further samples were collected from other departments beside the surgical department as further sources of *P. aeruginosa* infection. Moreover, it would be more conclusive, if we investigated larger number of patient samples for better statistical analysis.

Conflict of interest

The authors report no conflicts of interest.

Authors' Contribution

All authors contributed equally to this work.

References

1-**Sen CK, Gordillo GM, Roy S, Kirsner R, Lambert L, Hunt TK, et al.** Human skin wounds: a major and snowballing threat to public health and the economy. Wound Repair and Regeneration 2009; 17(6): 763-771.

2-**Fournier A, Voirol P, Krähenbühl M, Bonnemain CL, Pantet O, Pagani JL.** Antibiotic consumption to early detect epidemics of Pseudomonas aeruginosa in a burn center: a paradigm shift in the epidemiological surveillance of Pseudomonas aeruginosa

nosocomial infections. Burns 2015; 42(3): 564-542.

3-**Van Delden C, Iglewski BH.** Cell-to-cell signaling and Pseudomonas aeruginosa infections. Emergency Infectious Diseases 1998; 4(4): 551–560.

4-**Muller M, Maitz PK.** Pseudomonas pyocyanin inhibits wound repair by inducing premature cellular senescence: role for p38 mitogen-activated protein kinase. Burns 2009; 35(4): 500–508.

5-**Ikpeme EM, Enyi-Idoh KH, Nfongeh JF, Etim LB, Akubuenyi FC**. Prevalence, antibiogram profile and cross transmission of Pseudomonas aeruginosa in a tertiary burn unit, Malaysian Journal of Microbiology 2013; 9(1): 116-119.

6-**Greer N, Foman NA, MacDonald R, Dorrian J, Fitzgerald P, Rutks I, et al.** Advanced wound care therapies for non-healing diabetic, venous, and arterial ulcers: a systematic review. Annals of Internal Medicine 2013; 159(8): 532-542.

7-**Veesenmeyer JL, Hauser AR, Lisboa T, Rello J.** Pseudomonas aeruginosa virulence and therapy: evolving translational strategies. Critical Care Medicine 2009; 37(5): 1777-1786.

8-**Neish AS.** Microbes in gastrointestinal health and disease. Gastroenterology 2009; 136(1): 65–80.

9-**Aarti C, Martina C, Khusro A.** Antimycobacterial, anticancer, and antiviral properties of probiotics: An overview. Microbes and Infectious Diseases 2020. doi: 10.21608/mid.2020.34124.1029.

10-**Pradhan D, Singh R, Tyagi A, Rashmi H, Batish VK, Grover S**. Assessing the safety and efficacy of Lactobacillus plantarum MTCC 5690 and Lactobacillus fermentum MTCC 5689 in colitis mouse model, Probiotics Antimicrobial Proteins 2019; 11: 910–920**.**

11-**Song L, Xie W, Liu Z, Guo D, Zhao D, Qiao X, et al.** Oral delivery of a Lactococcus lactis strain secreting bovine lactoferricin–lactoferrampin alleviates the development of acute colitis in mice. Applied Microbiological Biotechnology 2019; 103: 6169–6186.

12-**Sun M, Zhang F, Yin X, Cheng B, Zhao C, Wang Y, et al.** Lactobacillus reuteri F-9-35 prevents DSS-induced colitis by inhibiting pro-inflammatory gene expression and restoring the gut microbiota in mice. Journal Food Science 2018; 83: 2645–2652.

13-**Alexandre Y, Le Blay G, Boisramé-Gastrin S, Le Gall F, Héry-Arnaud G, Gouriou S, et al.** Probiotics: A new way to fight bacterial pulmonary infections? Médecine et Maladies Infectieuses 2014; 44(1): 9-17.

14-**Das JK, Mishra D, Ray P, Tripathy P, Beuria TK, Singh N, et al**. In vitro evaluation of anti-infective activity of a Lactobacillus plantarum strain against Salmonella enterica serovar Enteritidis. Gut Pathogens 2013; 5(1):1-1.

15-**Ramos AN, Sesto Cabral ME, Noseda D, Bosch A, Yantorno OM, Valdez JC.** Anti-pathogenic properties of Lactobacillus plantarum on Pseudomonas aeruginosa: the potential use of its supernatants in the treatment of infected chronic wounds. Wound Repair and Regeneration 2012; 20(4): 552-562.

16-**Cheesbrough M.** Tropical countries. Part 2. 2nd ed. London: Cambridge University Press. 2007; 7: 234-237.

17-**Kurachi M.** Studies on the Biosynthesis of Pyocyanin (II): Isolation and determination of pyocyanin 1958; 36(6):174-87.

18-**Elsheikh LE, Bergman RU, Cryz JR SJ, Wretlind BE.** A comparison of different methods for determining elastase activity of

Pseudomonas aeruginosa strains from mink. Acta Pathologica Microbiologica Scandinavica Series B: Microbiology 1986;94(1-6):135-8.

19-**Meng F, Zhao H, Nie T, Lu F, Zhang C, Lu Y, et al.** Acetate activates bacteriocins synthesis of Lactobacillus by controlling quorum sensing. Applied and Environmental Microbiology 2021: AEM-00720.

20-**Lv X, Cui T, Du H, Sun M, Bai F, Li J, et al.** Lactobacillus plantarum CY 1-1: A novel quorum quenching bacteria and anti-biofilm agent against Aeromonas sobria. LWT 2021; 137:110439.

21-**Rana S, Bhawal S, Kumari A, Kapila S, Kapila R**. pH-dependent inhibition of AHL-mediated quorum sensing by cell-free supernatant of lactic acid bacteria in Pseudomonas aeruginosa PAO1. Microbial pathogenesis 2020; 142:104105.

22-**Al-Madboly LA, Abdullah AK.** Potent antagonistic activity of Egyptian Lactobacillus plantarum against multiresistant and virulent food-associated pathogens. Frontiers in Microbiology 2015; 6.

23-**Chang CY, Krishnan T, Wang H, Chen Y, Yin WF, Chong YM, et al.** Non-antibiotic quorum sensing inhibitors acting against N-acyl homoserine lactone synthase as druggable target. Scientific reports 2014; 4: 7245.

24-**Valdez JC, Peral MC, Rachid M, Santana M & Perdigon G.** Interference of Lactobacillus plantarum with Pseudomonas aeruginosa in vitro and in infected burns: the potential use of probiotics in wound treatment. Clinical Microbiology and Infection 2005; 11(6): 472-479.

Shams Eldeen MA, Elnahal A, ElSawaf M, Ads A, Samy SM. Effect of *Lactobacillus plantarum* on virulence factors of *Pseudomonas aeruginosa* isolated from wound infection. Microbes Infect Dis 2021; 2(4): 790-796.