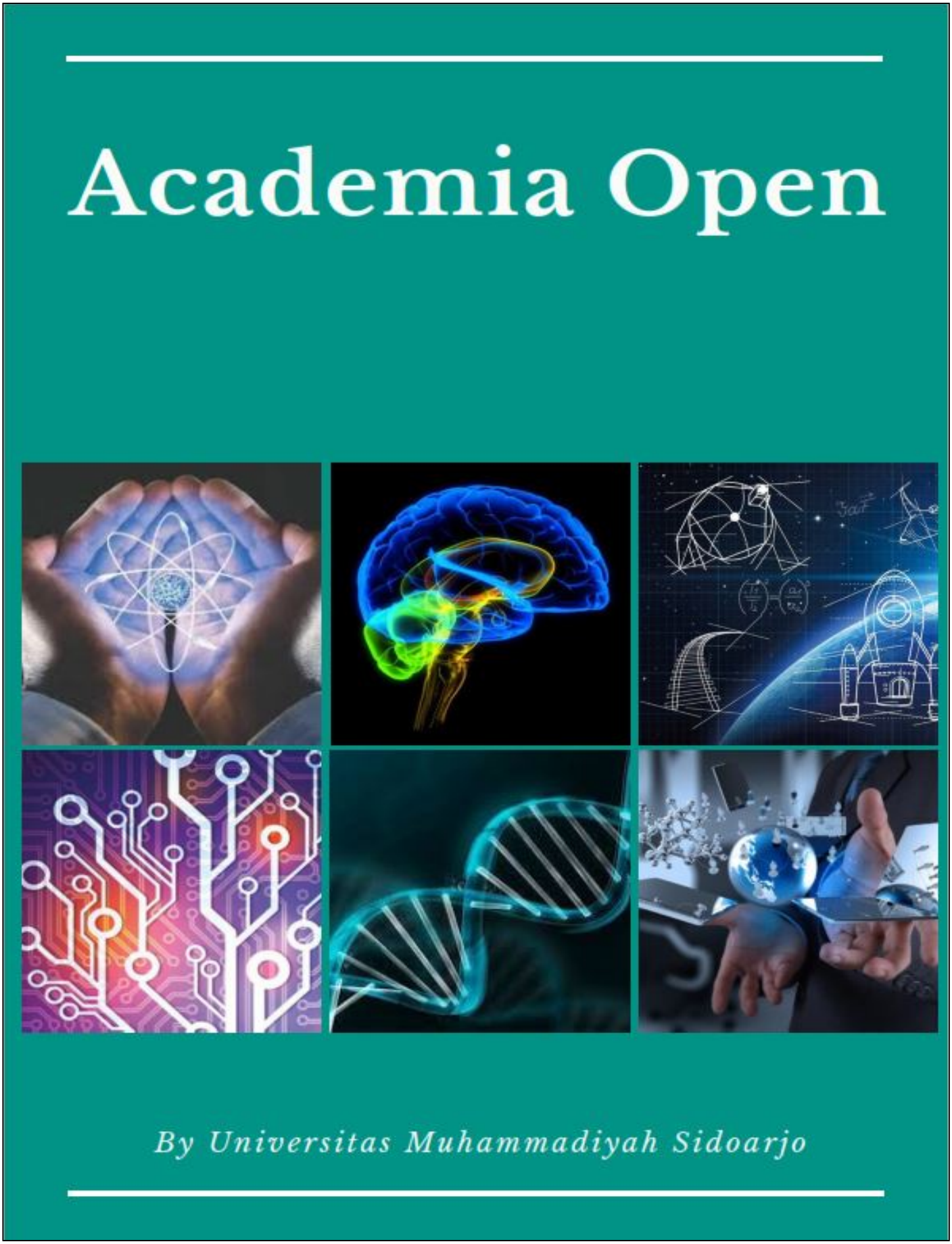
Academia Open
Vol 9 No 2 (2024): December
DOI: 10.21070/acopen.9.2024.10289 . Article type: (Microbiology)

Table Of Content

Journal Cover 2
Author[s] Statement 3
Editorial Team 4
Article information 5
Check this article update (crossmark) 5
Check this article impact 5
Cite this article 5
Title page 6
Article Title 6
Author information 6
Abstract 6
Article content 7

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Academia Open
Vol 9 No 2 (2024): December
DOI: 10.21070/acopen.9.2024.10289 . Article type: (Microbiology)



ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Academia Open
Vol 9 No 2 (2024): December
DOI: 10.21070/acopen.9.2024.10289 . Article type: (Microbiology)

Originality Statement

The author[s] declare that this article is their own work and to the best of their knowledge it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the published of any other published materials, except where due acknowledgement is made in the article. Any contribution made to the research by others, with whom author[s] have work, is explicitly acknowledged in the article.

Conflict of Interest Statement

The author[s] declare that this article was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright Statement

Academia Open
Vol 9 No 2 (2024): December
DOI: 10.21070/acopen.9.2024.10289 . Article type: (Microbiology)

EDITORIAL TEAM

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Academia Open
Vol 9 No 2 (2024): December
DOI: 10.21070/acopen.9.2024.10289 . Article type: (Microbiology)

Article information

Check this article update (crossmark)

Check this article impact (*)

Save this article to Mendeley

(*) Time for indexing process is various, depends on indexing database platform

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Academia Open
Vol 9 No 2 (2024): December
DOI: 10.21070/acopen.9.2024.10289 . Article type: (Microbiology)

Interference Between S. Aureus and P. Aeruginosa Clinical Isolates

Interferensi Antara S. Aureus dan P. Aeruginosa Isolat Klinis

Aghadeer Sarmad Najeeb, akaders.n.321@gmail.com, (1)
Baqubah teaching hospital/ Diyala province/ Iraq, Iraq

Nagham Mohamed Khalaf , nagham.m@uodiyala.edu.iq, (0)
Diyala University/ Biology Department, Iraq

Israa Salim Kamil, israa@gmail.com, (0)
Baqubah teaching hospital/ Diyala province/ Iraq, Iraq

(1) Corresponding author

Abstract

General Background: Understanding microbial interactions between Pseudomonas aeruginosa and Staphylococcus aureus is crucial for clinical infections, as they often coexist and influence each other's growth and virulence. **Specific Background:** Both organisms are known for their ability to form biofilms and exhibit multidrug resistance (MDR), complicating treatment strategies in hospitalized patients. **Knowledge Gap:** Previous studies have explored the virulence of P. aeruginosa and S. aureus, but there is limited understanding of their direct in vitro interactions, particularly in protease and lipase production. **Aims:** The study examined the interaction between P. aeruginosa and S. aureus isolates in hospitalized patients' sputum, urine, and blood samples, focusing on virulence factors, biofilm formation, and antibiotic resistance patterns. **Results:** In Diyala, Iraq, 50 clinical isolates showed P. aeruginosa as protease producers, lipase producers, and biofilm producers, with significant MDR phenotypes in both species. **Novelty:** This study highlights the ability of *P. aeruginosa* to produce staphylolysin, offering novel insights into the antagonistic mechanisms that may suppress *S. aureus* in co-infections. **Implications:** The study emphasizes the significance of understanding microbial interactions in clinical infections, particularly in biofilm-associated MDR infections, to improve treatment outcomes and guide more effective therapeutic approaches.

Highlights:

1. *P. aeruginosa* staphylolysin inhibits *S. aureus* growth in vitro.
2. Both bacteria produce biofilms and exhibit multidrug resistance.
3. Microbial interactions impact infection severity and treatment strategies.

Keywords: Microbial interactions, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Biofilm, Multidrug resistance

Published date: 2024-10-14 00:00:00

Academia Open
Vol 9 No 2 (2024): December
DOI: 10.21070/acopen.9.2024.10289 . Article type: (Microbiology)

Introduction

Pseudomonas aeruginosa is a common bacterium found in the environment. It is currently among the most clinically significant opportunistic infections. It is a significant nosocomial pathogen that has been linked to numerous serious opportunistic infections, particularly in individuals with impaired immune systems (Abd Al-Mayali and Salman, 2020). The presence of many virulence factors, including biofilm formation, is necessary for P. aeruginosa to cause a broad range of illnesses. Biofilms are collections of one or more species of bacteria that adhere to either living or non-living surfaces (Da Costa et al., 2021). P. aeruginosa's pathogenesis is significantly influenced by the bacteria's capacity to form biofilms on both types of surfaces (Saffari et al., 2017).

One of the most significant harmful microbes implicated in infections acquired in hospitals and the community is Staphylococcus aureus (Miller et al., 2020). It has been and still is a primary cause of hospital-acquired infections as well as a major cause of mortality. In addition to causing community infections, it encompasses several illnesses like epidural abscess, urinary tract infections (UTI), toxic shock syndrome, otitis media, and meningitis in addition to numerous clinical infections like bacteremia, osteoarthritis, pneumonia, skin infections, and infective endocarditis (Rasheed et al., 2020). Numerous virulence factors, including hemolysins, surface protein adhesins, enterotoxins, and other substances that promote the start of the disease process, immune evasion, and tissue destruction are produced by S. aureus and are known to influence the kind and intensity of staphylococcal infections (Cheung et al., 2021).

Bacterial capacity to form biofilm plays a major role in pathogenicity. S. aureus forms structure of a complex extracellular biofilm which affords a fully safe and practical habitat for progress, feeding, and recolonization of microcolonies by sessile cells after their spread. Biofilm protect cells of S. aureus from oppositive circumstances, for instance changes in temperature, nutrient shortages, and desiccation, and most prominently, it keeps the cells from antibiotics effects (Idrees et al., 2021). Medicines are increasingly becoming partially or completely impractical against S. aureus since antibiotics are incapable to pierce biofilm round bacterial cells. This increased tolerance to antibiotics has participated to escalate and expansion of antibiotic resistance. Biofilms also play other roles, such as evasion of host’s innate immune system leading to chronic infections and ability to adapt through gene progression and switch of genetic material (Idrees et al., 2021). This study aimed to isolation, detecting some virulence factors and establish the interference between the isolates under study.

Methods

Isolation of P. aeruginosa and S. aureus

Following collection, the samples were grown on blood and MacConkey agar and incubated for 24 hours at 37°C. The growing bacterial colonies were moved to mannitol salt agar and cetrimide agar, where they were cultured for 24 hours at 37°C. The isolates that carrying P. aeruginosa and S. aureus attributes then picked up for further confirmatory biochemical tests and VITIK2 to obtain pure isolates.

Identification of isolates under study

Identification of P. aeruginosa and S. aureus were carried out using of morphological, biochemical and VITEK2 confirmatory tests:

The isolates were diagnosed initially based on their capability to grow on MacConkey and blood agar. They were cultivated in order to determine the culture characteristics, such as hemolysis on blood agar, growth on mannitol salt agar, color and odor of produced pigment, lactose fermentation, which showed up as pink colonies, and non-lactose fermentation, which showed up as pale colonies (Baron et al, 2007).

Phenotypic detection of virulence factors:

Biofilm production assays

In order to test for biofilm formation, the Microliter Plate Assay was carried out using the technique outlined by Babapour et al. (2016).

Antibiotic sensitivity test

Test the antibiotic sensitivity had been performed for all isolates by VITEK2 system.

Protease production

A regular drilling done in Skim milk agar medium was previously prepared and inoculated by bacterial suspension. The appearance of inhibition zone around the hall refers to positive results (Benson, 2002).

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Staphylolysin production

A tryptic soy agar containing 0.2% (weight/volume) S. aureus that isolated and killed by heat (100°C for 10 min.) has been inoculated with P. aeruginosa isolates and incubated at 37°C for 24hrs. The diameter of inhibition zone around the growth was measured (Diggle et al., 2002).

Hemolysin production

Loopful of each isolate was streaked on blood agar base with 5% blood and incubated at 37ºC for 24 hr. The formation of a clear colorless area due to hemolysis of red cells signified hemolysin production (β- hemolysis) (Benson, 2002).

Lipase production

Bacterial colonies were inoculated on Egg yolk agar with then incubated at 37ºC for 24 hr. Appearance of lipolytic zone refers to positive results (Okwu1 et al., 2014).

Result and Discussion

Result

Isolate and Identified of P. aeruginosa and S. aureus

P. aeruginosa bacterial colonies appeared pale, colorless, with irregular rims on MacConkey agar due to the non-fermentation of lactose sugar, and were characterized by their odor resembling the smell of fermented grapes. On the blood agar appeared gray in color, completely β-hemolysis, The isolates also showed clear growth on selective cetrimide agar medium, which contains Cetrimide (0.03%). When S. aureus is identified on blood agar, the colonies generate ß-hemolysin, which is produced in a clear, transparent region around the colonies. A selective differential growth media is mannitol salt agar. Yellow colonies are produced by S. aureus. biochemical test results are shown in table (1).

Tests	P. aeruginosa	S. aureus
Catalase	+	+
Oxidase	+	-
Coagulase	-	+
Motility	Motile	Non-motile
Blood hemolysis	β- hemolysis	β- hemolysis
Gram stain	-	+
Pigments production	V	-
Protease	100 %	65%
Lipase	91%	88%

Table 1. *Biochemical tests and virulence factors for P. aeruginosa identification*

+ = positive, - = negative

Fifty (20%) S. aureus and P. aeruginosa isolates were identified among 250 different clinical samples (wounds, burns, urine, blood and ear) after conducting phenotypic, microscopic, and biochemical testing during the research period (January – July/ 2024) to confirm their diagnosis.

Virulence factors

The isolates were investigated for their possession of virulence factors including hemolysin production, proteases (total proteases and staphylolytic activity of elastase A), lipase and biofilm production.

In this study, the range for production of each virulence factor was arbitrarily selected and isolates were categorized as low or high producer (Table 1 and 2).

Biofilm production

In comparison to the negative control, Table (2), the results demonstrated that 100% of all isolates produced biofilm to varying degrees. This conclusion is consistent with the findings of Hatem et al.'s study (2021), which showed that every isolate of S. aureus was capable of producing a biofilm, as well as the findings of Al-draghi and

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Academia Open
Vol 9 No 2 (2024): December
DOI: 10.21070/acopen.9.2024.10289 . Article type: (Microbiology)

Saeed's study (2020), which showed that every isolate of P. aeruginosa was capable of producing a biofilm.

Biofilm	P. aeruginosa	S. aureus
Strong	24 (48%)	16 (32%)
Moderate	16 (32%)	21 (42%)
Weak	10 (20%)	13 (26%)
Total	50 (100%)	50 (100%)

Table 2. *formation of biofilm results*

Antibiotics resistance results

The VITEK2 system was utilized to establish sensitivity to antibiotic of S. aureus and P. aeruginosa isolates utilizing varying antibiotics. Figures 1 and 2 included the following results:

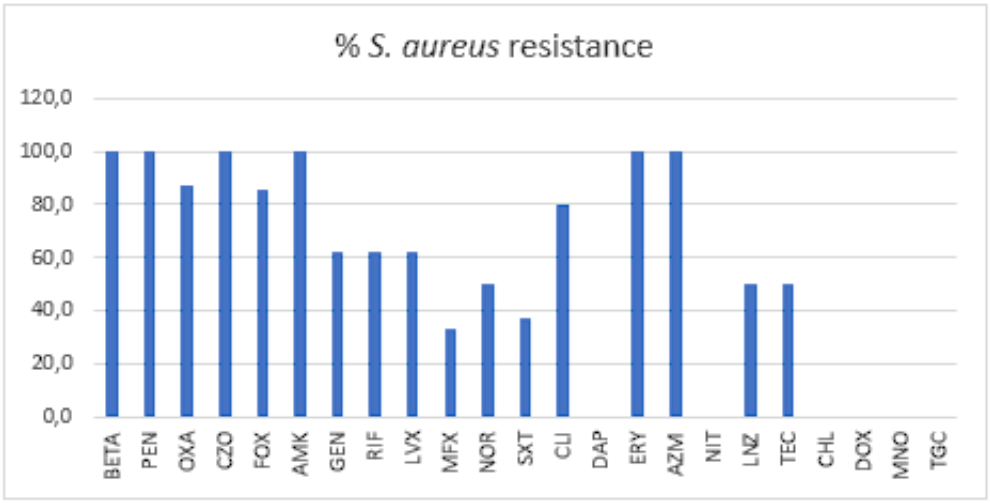


Figure 1. *Antibiotic resistance pattern of S. aureus*

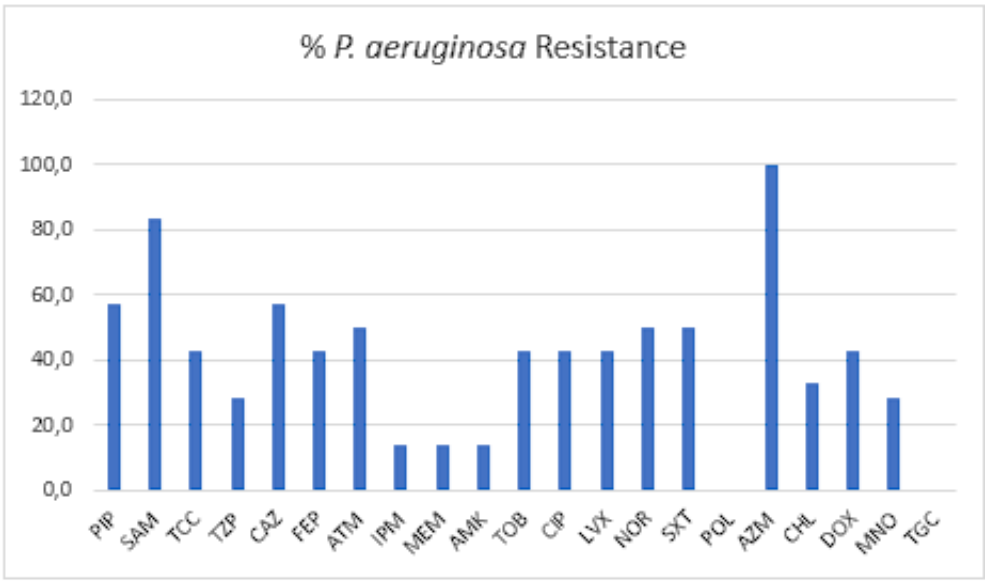


Figure 2. *Antibiotic resistance pattern of P. aeruginosa*

Staphylolysin production

Pseudomonas aeruginosa isolates were subjected to semi-quantitative screening by growing them on the tryptic soy agar medium + 0.2% (weight / volume) of the heat-killed S. aureus bacteria to investigate the susceptibility of

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

isolates to the production of staphylolycin enzyme, depending on the appearance of the translucent area around their colonies, with transparent halo diameters ranging from 6 - 21 mm. The results showed that 20 isolates (40%) of the isolates under study produced the enzyme but different in their production ability. Except for Lomholt et al (2001) and Najim 2009, few literatures on P. aeruginosa recorded an information on its production.

Discussion

Healthcare specialists should be aware of the growing prevalence of nosocomial microbial resistance and suitable antibiotics usage to treat such infections, as P. aeruginosa is one of the most recurrent nosocomial pathogens to be isolated in hospitals (Ekrem & Rokan, 2014).

Antibiotic resistance arises from evolution under the pressure of natural selection, which is triggered by the presence of antibiotics in the bacterial environment. Despite their adaptability, bacteria can only develop resistance to a restricted range of antibiotics through a variety of mechanisms, such as altering the target molecule to render it indecipherable to the antibiotic, producing bacterial enzymes that modify the antibiotic molecule, decreasing the permeability of bacterial cells, or developing active efflux systems to thwart antibiotic takeover. Concurrent application of the aforementioned methods may lead to a phenotype resistant to particular kinds of antibiotics. However, the emergence of antibiotic-resistant strains is due to alterations in chromosomal DNA (vertical evolution) or the introduction of foreign genetic material (horizontal evolution) (Manu et al., 2011).

Biofilm formation by the bacterial isolates under study is one of the most widespread virulence factors, because it indicates the presence of bacteria in contaminated and pathogenic environments (Holá et al., 2006). The biofilm works to provide protection against host immune defenses, and thus works to concentrate nutrients and protect them from antibiotics and phagocytic cells (Garofalo et al., 2007).

The significant proportion of biofilm-producing isolates in the current study may account for the high level of antibiotic resistance of the isolates. In order to determine the best course of treatment, it is critical to know the concentration of antibiotic needed to stop the growth of the bacteria that make up the biofilm. This is because standard antibiotic concentrations kill the cells surrounding the biofilm, while the cells inside the polysaccharide remain intact and serve as a center for periodic re-growth and re-infection (Saxena et al., 2014).

Results of the study showed high resistance of P. aeruginosa isolates to Cephalosporins, Monobactam, Penicillins, Fluuroquinolones, and Cephemes. This is due to the ability of bacteria to produce beta-lactam enzymes such as broad-spectrum β-lactamase enzymes (ESβLs), which work to degrade penicillin and cephalosporins in particular, and whose genes are either on chromosomes or on plasmids in many Types of bacteria, which leads to multiple resistance to target proteins to different antibiotics (Abdullah and Mahdi, 2016).

The main reason for resistance to aminoglycosides is the presence of modified aminoglycosidase enzymes (AMEs). The high rate of resistance to aminoglycosides is due to the indiscriminate use of these antibiotics (Haldorsen, 2011). Fluoroquinolones prevent the construction of bacterial DNA by inhibiting the enzyme DNA gyrase, and thus inhibiting the transcription of DNA. Nuclear and its reproduction. Resistance of P. aeruginosa to this group of antibiotics is due to a mutation in the target enzyme, DNA gyrase, or via the efflux pump system (Aghazadeh et al., 2014).

The widespread and indiscriminate use of these antibiotics by patients (in many cases) may lead to the emergence of resistance due to the availability, ease of oral administration, and cheap price, as the high rate of bacterial resistance in general to antibiotics is a man-made problem with a global spread, but it is evident in developing countries (Laxmi and Sarita, 2014).

Conclusion

The pathogenicity of P. aeruginosa and S. aureus is supported by a number of virulence characteristics, including virulence and antibiotic resistance. Among clinical isolates of P. aeruginosa, there is a substantial correlation between biofilm development and drug susceptibility profile. The ability to produce biofilms showed a discernible tendency toward the MDR resistance phenotype. P. aeruginosa is able to create staphylolysin, which stops S. aureus from growing

References

1. . M. Abd Al-Mayali and E. D. Salman, "Bacteriological and molecular study of fluoroquinolones resistance in Pseudomonas aeruginosa isolated from different clinical sources," Iraqi Journal of Science, vol. 61, no. 11, pp. 2204-2214, 2020.
2. . M. Aghazadeh, Z. Hojabri, R. Mahdian, M. R. Nahaei, M. Rahmati, T. Hojabri, and O. Pajand, "Role of efflux pumps: MexAB-OprM and MexXY (-OprA), AmpC cephalosporinase and OprD porin in non-metallo-β-lactamase producing Pseudomonas aeruginosa isolated from cystic fibrosis and burn patients," Infection,

Genetics and Evolution, vol. 24, pp. 187-192, 2014.
3. . W. A. H. Al-Draghi and R. A. A. K. Saeed, "Synergistic effect of amikacin and ciprofloxacin on pelA and algD genes in Pseudomonas aeruginosa," Indian Journal of Forensic Medicine & Toxicology, vol. 14, no. 4, pp. 1608-1614, 2020.
4. . E. Babapour, A. Haddadi, R. Mirnejad, S. A. Angaji, and N. Amirmozafari, "Biofilm formation in clinical isolates of nosocomial Acinetobacter baumannii and its relationship with multidrug resistance," Asian Pacific Journal of Tropical Biomedicine, vol. 6, no. 6, pp. 528-533, 2016.
5. . E. J. Baron, S. M. Finegold, and I. L. R. Peterson, Bailey and Scott's Diagnostic Microbiology, 9th ed. St. Louis, MO: Mosby, 2007.
6. . H. G. Benson, Microbiological Applications: Laboratory Manual in General Microbiology, 8th ed. New York: McGraw-Hill, 2002.
7. . G. Y. C. Cheung, J. S. Bae, and M. Otto, "Pathogenicity and virulence of Staphylococcus aureus," Virulence, vol. 12, no. 1, pp. 547-569, 2021.
8. . D. Da Costa, C. Exbrayat-Héritier, B. Rambaud, S. Megy, R. Terreux, B. Verrier, and C. Primard, "Surface charge modulation of rifampicin-loaded PLA nanoparticles to improve antibiotic delivery in Staphylococcus aureus biofilms," Journal of Nanobiotechnology, vol. 19, pp. 1-17, 2021.
9. . S. P. Diggle, K. Winzer, A. Lazdunski, P. Williams, and M. Camara, "Advancing the quorum in Pseudomonas aeruginosa: mvaT and the regulation of N-acetyl homoserine lactone production and virulence gene expression," Journal of Bacteriology, vol. 184, no. 10, pp. 2576-2586, 2002.
10. . K. Ekrem and D. K. Rokan, "Antibiotic susceptibility patterns of Pseudomonas aeruginosa strains isolated from various clinical specimens," Sky Journal of Microbiology Research, vol. 2, no. 2, pp. 13-17, 2014.
11. . C. K. Garofalo, T. M. Hooton, S. M. Martin, W. E. Stamm, J. J. Palermo, J. I. Gordon, and S. J. Hultgren, "Escherichia coli from urine of female patients with urinary tract infections is competent for intracellular bacterial community formation," Infection and Immunity, vol. 75, no. 1, pp. 52-60, 2007.
12. . B. C. Haldorsen, "Aminoglycoside resistance in clinical gram-negative isolates from Norway," M.S. thesis, Universitetet i Tromsø, Tromsø, Norway, 2011.
13. . Z. A. Hatem, S. A. Jasim, and Z. H. Mahdi, "Phenotypic and genotypic characterization of antibiotic resistance in Staphylococcus aureus isolated from different sources," Jundishapur Journal of Microbiology, vol. 14, no. 4, pp. 1-10, 2021.
14. . V. Holá, F. Ruzicka, and M. Votava, "The dynamics of Staphylococcus epidermidis biofilm formation in relation to nutrition, temperature, and time," Scripta Medica, vol. 79, no. 3, pp. 169-174, 2006.
15. . M. Idrees, S. Sawant, N. Karodia, and A. Rahman, "Staphylococcus aureus biofilm: Morphology, genetics, pathogenesis, and treatment strategies," International Journal of Environmental Research and Public Health, vol. 18, pp. 7602, 2021.
16. . M. Laxmi and G. B. Sarita, "Diversity characterization of biofilm forming microorganisms in food sampled from local markets in Kochi, Kerala, India," International Journal of Recent Scientific Research, vol. 5, pp. 1070-1075, 2014.
17. . D. Manu, I. Lupan, and O. Popescu, "Mechanisms of pathogenesis and antibiotics resistance in Escherichia coli," Annals of the Romanian Society for Cell Biology, vol. 16, no. 2, pp. 45-57, 2011.
18. . L. S. Miller, V. G. Fowler Jr., S. K. Shukla, W. E. Rose, and R. A. Proctor, "Development of a vaccine against Staphylococcus aureus invasive infections: Evidence based on human immunity, genetics and bacterial evasion mechanisms," FEMS Microbiology Reviews, vol. 44, no. 1, pp. 123-153, 2020.
19. . M. U. Okwu, O. Mitsan, and O. P. Okeke, "Prevalence and antimicrobial susceptibility profiles of community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA) isolates among healthy individuals in Okada, South-South, Nigeria," Pharmaceutical, Biological & Chemical Sciences Journal, vol. 1, no. 1, pp. 1-9, 2014.
20. . T. H. Rasheed, J. K. Luti, and A. M. Alaubydi, "Purification and characterization of bacteriocin from Lactobacillus acidophilus HT1 and its application in a cream formula for the treatment of some skin pathogens," Iraqi Journal of Agricultural Sciences, vol. 51, no. 5, pp. 1381-1393, 2020.
21. . M. Saffari, S. Karami, F. Firoozeh, and M. Sehat, "Evaluation of biofilm-specific antimicrobial resistance genes in Pseudomonas aeruginosa isolates in Farabi Hospital," Journal of Medical Microbiology, vol. 66, no. 7, pp. 905-909, 2017.
22. . S. Saxena, G. Banerjee, R. Garg, and M. Singh, "Comparative study of biofilm formation in Pseudomonas aeruginosa isolates from patients of lower respiratory tract infection," Journal of Clinical and Diagnostic Research, vol. 8, no. 5, pp. DC09-DC11, 2014.

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo