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Interference Between *S. Aureus* and *P. Aeruginosa* Clinical Isolates

Interferensi Antara S. Aureus dan P. Aeruginosa Isolat Klinis

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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:

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

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

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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 opposite 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 well refers to positive results (Benson, 2002).

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 37°C 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 37°C 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 β -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	<i>P. aeruginosa</i>	<i>S. aureus</i>
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

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

Biofilm	<i>P. aeruginosa</i>	<i>S. aureus</i>
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:

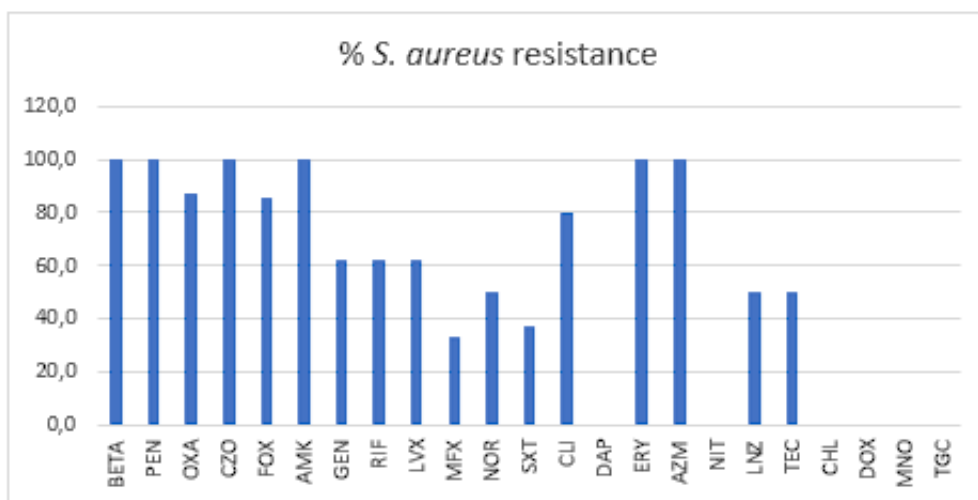


Figure 1. Antibiotic resistance pattern of *S. aureus*

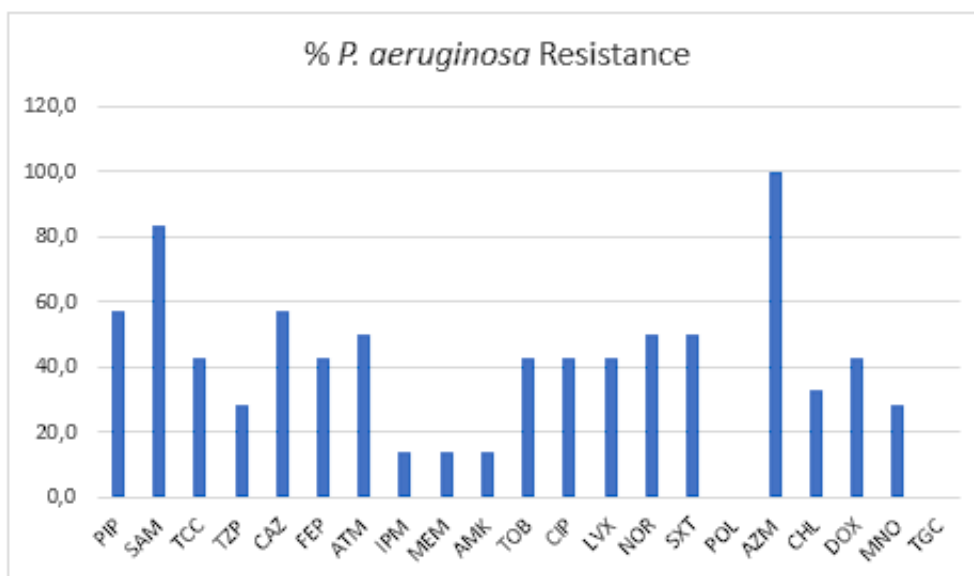


Figure 2. Antibiotic resistance pattern of *P. aeruginosa*

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

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, Fluoroquinolones, and Cephemes. This is due to the ability of bacteria to produce beta-lactam enzymes such as broad-spectrum β -lactamase enzymes (ES β LS), 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

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