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ORIGINAL RESEARCH ARTICLE

Impact of indole against persister cell *Pseudomonas aeruginosa* isolates

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ABSTRACT

Background: Because of their metabolic dormancy and antibiotic resistance, persister cells pose a serious threat to the treatment of chronic infections.

Objective: The antibacterial activity of indole against *Pseudomonas aeruginosa* persister isolates is examined in this work.

Methods: In Baghdad, Iraq, fifty clinical isolates of *P. aeruginosa* were gathered from different sources. A quick killing technique was used to identify persister cells. The agar well diffusion method was used to assess indole's antibacterial activity, and the microtiter plate method with resazurin dye was used to calculate the Minimum Inhibitory Concentration (MIC).

Result: Five (10%) of the fifty isolates were identified as persisters. With inhibition zones spanning from 25 to 37 mm, indole demonstrated strong antibacterial action. It was discovered that the MIC values for indole against these persistent isolates ranged from 156.25 to 312.5 µg/ml.

Conclusion: The results show that indole is a promising antibacterial drug that can target persister cells of *P. aeruginosa*. It is advised to conduct an additional study to investigate the underlying processes of this action in order to create innovative treatment approaches for chronic bacterial infections that are chronic.

Keywords: Antibacterial activity; Biofilm; Indole; Persister.

1. INTRODUCTION

Antibiotic resistance is an increasing concern in the global medical community, with bacteria capable of rejecting both old and novel medicines in a variety of ways [1]. This is attributable to innate (innate), acquired (horizontal gene transfer), or adaptive (biofilm and persister formation) resistance [2]. Persister cells are a non-dividing subset of cells that have demonstrated multidrug resistance and can withstand treatment with all known antimicrobials as well as stress, by entering a dormant state in which cellular mechanisms are inactive. Because no mutations occur, Persisters act like wild-type cells [3]. Stresses such as food deprivation, oxidative stress, or the presence of antibiotics at sub-lethal doses cause persister cells to form. Bacterial Persister cells rebel, do not grow or die when exposed to bactericidal drugs, and wake up from a metabolically quiescent condition when no bactericidal agents are present, demonstrating remarkable multidrug resistance [4]. Persister cells display multidrug resistance because dormancy is a primary survival mechanism for bacteria, and antibiotics are often only effective against actively developing bacteria. Persister cells do survive

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antibiotic treatments, whether they are single or numerous [5]. Persistent pathogens generate repeated infections that lead to chronic infectious illnesses, leading to drug usage and diminished antibiotic effectiveness. As a result, in vivo investigations on persisters are crucial for the sustained use of currently available antibiotics [6]. So, to eradicated these difficult infections they needed to new approach to fight persister cell infection of *Pseudomonas aeruginosa*, one of these anti persister strategies done by using Indole and/or its derivatives, Indole is an aromatic heterocyclic organic compound, indole exhibited anti-inflammatory, analgesic, anti-microbial, anti-convulsant, antidepressant, anti-diabetic, anthelmintic, and anti-allergic characteristics, among other biological qualities [7].In recent years, it has been reported that indole, its bioisosters, and derivatives have antimicrobial activity against Gram-negative bacteria, and the yeast *Candida albicans*, with antimicrobial activity against *Enterobacter*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis* in particular [8]. A recent study shows the synergistic effect of combining indole with antibiotics such as ciprofloxacin against persister cell of *K. pneumonia* [9]. Therefore, the present study aims to investigate the antibacterial effect of indole against *Pseudomonas aeruginosa* isolates.

2. MATERIALS AND METHODS

2.1. Bacterial isolates and growth conditions

Fifty, *Pseudomonas aeruginosa* isolates from different clinical sources, 19 isolates from sputum source, 15 isolates from burn source,11 isolates from urine source and 5 isolates from blood source were obtained from different hospitals in Baghdad, Iraq. All isolates were identified by using the biochemical tests and the Vitek-2 system. All bacterial isolates were grown on Luria Bertani (LB, Difco Laboratories) agar plates or in LB broth at 37 °C for 18-24 hr.

2.2. Detection of persister cells

Using rabidly killing procedures and freshly cultured cells with lytic solutions, a test for identifying persister cells was established [10]. P. aeruginosa isolates were cultivated overnight at 37 °C in LB broth and then diluted to the McFarland standard of 0.5 McFarland bacterial suspension. Then, in a 10 mL sterile test tube, add 200 µL of buffer lysis solution, vortex mix well for 10 seconds, and then incubate at room temperature for 10 minutes. After that, 200 µL of the enzymatic lysis solution was gently mixed into the mixture. Finally, in a shaking incubator, incubate for 15 minutes at 200 rpm at 37 °C. A 10 µL suspension from the suspension spread on LB agar plates was used to determine the frequency of persister cells, which was subsequently incubated for 18-24 hours at 37°C.

2.3. Antibacterial activity and MIC of Indole

The agar well diffusion technique [11], was used to investigate the antibacterial activity of indole against persister *P. aeruginosa* isolates. On Muller Hinton agar plate, three wells with 8mm are made. The first well was filled with 100 µL of indole from stock solution, the other well filled by 100 µL of chemical solvent DMSO (dimethyl sulfoxide) used to see if they have any inhibition effects and the third well was filled with sterile distilled water, used as a negative control. And the second method, the minimum inhibitory concentration, was determined by using a 96-well polystyrene microtiter plate. Briefly, 100 µL Muller Hinton broth was put to micro titer plate for each well, then 100 µL indole from stock solution was added to the first well vertical row (A1-A10), followed by 1:2 serial dilutions. The last well's 100 µL were deserted. (A11-H11), considered as a positive control, they contain only M.H. broth and 5 µL of bacterial suspension. All wells (excluding negative control row A12-H12, which was solely filled with M.H broth only) were filled with 5 µL of persister *P.*

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aeruginosa bacterial dilution suspension (108 CFU/mL), then incubated for 24 hours at 37 °C. Finally, 10 µL of resazurin dye was added to all wells and incubated for 4 hours at 37 °C to be ready for reading. The actions of different concentrations of Indole for the bacterial growth were detected by using UV visible spectrophotometer [12].

3. RESULTS

3.1 Detection of persister cell formation

The detection of persister cell formation in *P. aeruginosa* isolates was tested by using a rapidly killing method. Results show that about of 50 of *P. aeruginosa* isolates, only five isolates were persister cells as shows in (Figure 1).

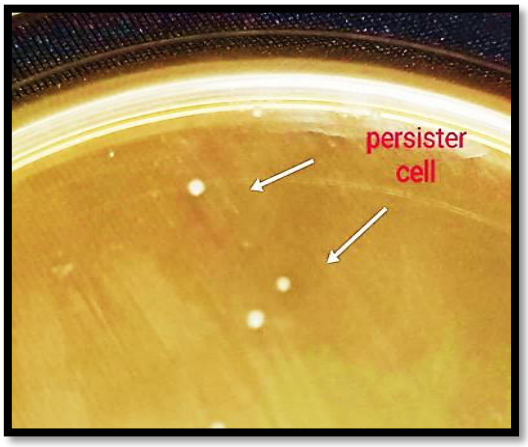
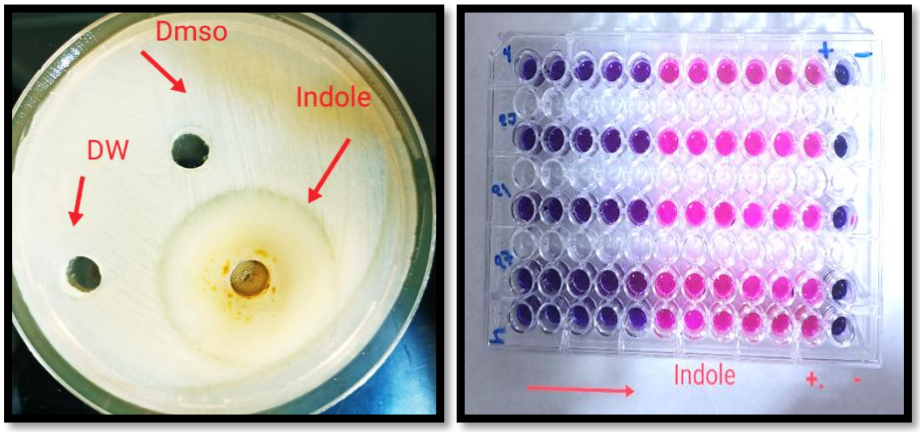


Figure 1. Persister cells formation in *P. aeruginosa* by a rapid killing method.

3.2 Antibacterial Activity and MIC of Indole

On persistence, indole was shown to have antibacterial efficacy against *P. aeruginosa*. In the agar well diffusion technique, indole demonstrated significant antibacterial activity, with inhibition zones ranging between 25 to 37 mm. while The MIC results of indole by using micro titer plate method showed a strong antibacterial activity range from 156.25 to 312.5 µg /ml as show in (Figure 2).



A **B**

Figure 2. Antibacterial activity and MIC of Indole by A- agar –well diffusion methods and B- micro titer plate

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4. DISCUSSION

The findings showed that indole has substantial antibacterial activity against *P. aeruginosa* persisters, with inhibition zones ranging from 25 to 37 mm. This robust effect is consistent with prior findings showing that indole derivatives can prevent biofilms and other harmful bacteria. The discovered MIC values (156.25-312.5 µg/ml) suggest that indole can be used as a strategic approach to combat chronic infections. These findings highlight the importance of additional study into persister development processes in order to create novel treatments and reduce antibiotic resistance. [13].

As a result, it's an ideal procedure that works without affecting the bacterial population size, strain, or physiological condition of the culture. Al-Timimi [14], from Baghdad, reported that two isolates of *K, pneumonia* can form persister cells by using a rapidly killing method. Treatment of persistent infections is difficult. Clinically, persisters underlie persistent and latent infections and post-treatment relapse, posing significant challenges in the treatment of many bacterial infections.

Studies have revealed that indole derivatives possess antimicrobial and biofilm-inhibiting activities against various pathogenic microorganisms, including *Escherichia coli* and *Pseudomonas aeruginosa* (15). Several bis-indole agents were demonstrated to exhibit activities against multidrug-resistant Gram-positive and Gram-negative bacterial species, including *A. baumannii* (16).

The study's focus on clinical isolates from a specific geographical area (Baghdad) may not represent *P. aeruginosa's* global genetic diversity. Furthermore, whereas indole shows strong in vitro activity, additional in vivo investigations are needed to assess its safety and efficacy in a biological system.

4. CONCLUSION

P.aeruginosa were identified exhibited persistent cell behavior. The results highlight the role of ibdole as an antibacterial agents.. further research on persister cell growth mechanisms is essential to develop novel treatments for persistent bacterial infections and minimize antibiotic resistance.

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CONFLICTS OF INTEREST

There were no conflicts of interest in this study.

AUTHOR CONTRIBUTIONS

All paper elements are created by the Author.

DATA AVAILABILITY STATEMENT

All data have been gathered in the biology department laboratory.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval for the study was secured from the Clinical Laboratory/Biology Department at the College of Science, Mustansiriyah University

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