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## **Comparison of doses of paracetamol or ibuprofen to inhibit the formation of biofilms *Pseudomonas aeruginosa* bacteria**

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**Abstract**--Background: Some antimicrobials are not effective for bacterial therapy Multiple drug resistance and that form biofilms so strategies of looking for alternative therapies began to be developed. Paracetamol and ibuprofen as non-antibiotic drugs that have the potential to inhibit the formation of biofilms. In this study, a trial was carried out comparing exposure to doses of paracetamol and ibuprofen to inhibit the formation of biofilms of clinical isolates stored *Pseudomonas aeruginosa*. Method: This study is a true experimental study. Results: The results of the study obtained paracetamol and ibuprofen were able to inhibit the formation of biofilms. A dose of paracetamol 1000mg is able to inhibit the formation of *Pseudomonas aeruginosa* biofilm up to 68.15%. A dose of ibuprofen 800mg is able to inhibit up to 49.13%. The results of statistical tests comparing doses capable of inhibiting, paracetamol and ibuprofen showed significant differences for all doses, except ibuprofen doses of 600mg and 800mg were not obtained significantly different from  $p=0.422$  values. Conclusion: Paracetamol and ibuprofen are able to inhibit the formation of biofilm of *Pseudomonas aeruginosa* bacteria with concentration-dependent activity through optical density value measurement tests using ELISA reader.

**Keywords**---Biofilm, Paracetamol, Ibuprofen, *Pseudomonas aeruginosa*.

## Introduction

The bacterium *Pseudomonas aeruginosa* has many virulence factors, one of which is biofilm, which makes *Pseudomonas aeruginosa* difficult to administer. Data in 2017 stated that bloodstream infections related to the formation of biofilms cause up to 15-30% of deaths (Pinto, Simões, & Borges, 2021). Some antimicrobials become ineffective for multiple drug resistance bacterial therapy and that forms biofilms, so strategies for finding therapeutic alternatives are beginning to be developed (Dai et al., 2019). It is now developing the drug as an antivirulence with target work on bacterial virulence factors and several strategies focus on regulation of virulence products through cell-to-cell communication barriers called quorum sensing. By inhibiting quorum sensing indirectly inhibits the formation of biofilms (Seleem et al., 2021). Antipyretics and analgesics are the most commonly used drugs in infectious diseases. Some analgesics directly and indirectly have the effect of inhibiting the formation of biofilms or antibiofilms. Previous studies have investigated that paracetamol has the potential to have virulence inhibitor activity and pathogenicity *Pseudomonas aeruginosa* strain PAO1 (Zimmermann & Curtis, 2017). Previous studies have proven that Non Steroidal Anti Inflammatory Drugs (NSAIDs) can be used as antimicrobials and antibiofilms in some bacteria since these drugs are increasingly used, so it is also expected to be able to treat biofilm related infections (Hall et al., 2016; Paes Leme & da Silva, 2021). In this study, researchers conducted a test by comparing exposure to doses of paracetamol and ibuprofen to inhibit the formation of biofilms of stored clinical isolate *Pseudomonas aeruginosa* in the Clinical Microbiology laboratory of Dr. Soetomo Regional General Hospital.

## Method

Strains: six isolates were stored in the clinical microbiology laboratory of Dr. Soetomo Regional General Hospital Surabaya which has been identified using the BD Phoenix™ semi-automatic system. Sampel research was taken by random sampling and salready went through preliminary tests to determine whether isolates have the ability to form biofilms or not. This research was approved by the Health Ethics Committee of Dr. Soetomo Regional General Hospital Surabaya based on an ethics certificate No. 0936/LOE/301.4.2/VI/2022. Chemical agents: Paracetamol and ibuprofen oral preparasion were from branded drugs which are often used as the main choice to reduce fever. Standard culture of bacteria for antibacterial assay was prepared in Triptic Soy Broth equivalent to a 0.5 MacFarland Nephelometer standard (reading to  $1 \times 10^8$  cfu/ml) ditambah dengan sukrosa 5%. To obtain stock solutions, paracetamol at doses of 125mg, 250mg, 500mg, 1000mg and ibuprofen 200mg, 400mg, 600mg, 800mg, each dissolved in DMSO solution. Each dose of paracetamol and ibuprofen took as much as 20  $\mu$ L added to the microplate well which already contained 20  $\mu$ L of *Pseudomonas aeruginosa* isolate solution and 180  $\mu$ L of Triptic Soy Broth. The microplate was incubated at  $35 \pm 2^\circ\text{C}$  for 1x24 hours. The next day the microplate contents are removed, wash them with 300  $\mu$ L of phosphate buffer saline (Ph 7.2) by 3 times.

Dry in the reverse position. The bacterial biofilm in the well is fixed with 150  $\mu\text{L}$  of 95% methanol in each well, let it stand for 20 minutes. The microplate is emptied by tapping tap on the base and leaving the greeting upside down. The biofilm was stained with 0.1% violet crystals for 15 minutes. Re-dry the microplate. Biofilms attached to the walls and base of the tube were dissolved with 300  $\mu\text{L}$  of ethanol (destaining) per well for 15 minutes. Close the microplate to prevent evaporation. Let stand at room temperature for 30 minutes. The optical density of biofilm formed is measured in thickness with the ELISA reader iMark<sup>TM</sup> Microplate Absorbance Reader Bio Rad with a wavelength of 595nm (Hassan et al., 2011; Balouiri, Sadiki, & Ibsouda, 2016; Haney et al., 2018; Haney, Trimble, & Hancock, 2021). Control by using 20  $\mu\text{L}$  isolate solution in Tryptic Soy Broth without the addition of paracetamol and ibuprofen. Statistical analysis: data were statistically analyzed by using one-way variance of analysis (ANOVA) dan grafik menggunakan statistical product and service solution (SPSS) version 21 dan GraphPad Prism Version 8.

## Discussion

There were eight concentration groups evaluated for biofilm formation adjusted to the treatment dose, each treatment was repeated 4 times. The results of the Optical density (OD) measurement of *Pseudomonas aeruginosa* biofilm against exposure to paracetamol and ibuprofen drugs are as follows:

Table 5.1  
Average Optical density and percentage of inhibition of *Pseudomonas aeruginosa* biofilm with exposure to paracetamol

Isolate <i>P. aeruginosa</i>	Positive control	PARACETAMOL (Average OD)			
		125mg	250mg	500mg	1000mg
1	0.163	0.094	0.082	0.065	0.045
Percentage decrease in OD		42.33%	49.69%	60.12%	72.39%
2	0.167	0.087	0.084	0.074	0.052
Percentage decrease in OD		47.90%	49.70%	55.69%	68.86%
3	0.153	0.118	0.098	0.074	0.053
Percentage decrease in OD		22.88%	35.95%	51.63%	65.36%
4	0.165	0.099	0.086	0.064	0.059
Percentage decrease in OD		40%	47.88%	61.21%	64.24%
5	0.162	0.098	0.086	0.073	0.045
Percentage decrease in OD		39.51%	46.91%	54.94%	72.22%
6	0.164	0.103	0.088	0.062	0.056
Percentage decrease in OD		37.19%	46.34%	62.19%	65.85%
Average OD	0.162	0.100	0.087	0.069	0.052
Average Percentage		38.30%	46.08%	57.63%	68.15%

Table 5.2  
Average Optical density and percentage of inhibition of *Pseudomonas aeruginosa* biofilm with exposure to ibuprofen

Isolate <i>P. aeruginosa</i>	positive control	IBUPROFEN (Average OD)			
		200mg	400mg	600mg	800mg
1	0.163	0.150	0.115	0.100	0.096
Percentage decrease in OD		7.97%	29.45%	38.65%	41.10%
2	0.167	0.144	0.119	0.082	0.080
Percentage decrease in OD		13.77%	28.74%	50.89%	52.09%
3	0.153	0.137	0.122	0.084	0.083
Percentage decrease in OD		10.46%	20.26%	45.09%	45.75%
4	0.165	0.139	0.112	0.083	0.081
Percentage decrease in OD		15.75%	32.12%	49.69%	50.90%
5	0.162	0.141	0.115	0.077	0.071
Percentage decrease in OD		12.96%	29.01%	52.47%	56.17%
6	0.164	0.139	0.136	0.093	0.084
Percentage decrease in OD		15.24%	17.07%	43.29%	48.78%
Average OD	0.162	0.142	0.120	0.087	0.083
Average percentage		12.69%	26.11%	46.68%	49.13%

From the results of this study, it can be seen that the highest inhibitory effect dose of Paracetamol, dose 1000mg, is able to inhibit up to 68.15% of the formation of *P. aeruginosa* biofilm and the highest dose of ibuprofen, dose 800mg, is able to inhibit only 49.13%, can be seen in Tables 5.1 and 5.2. Then visualized in the form of a graph as shown in figure 5.1,5.2,5.3, and 5.4.

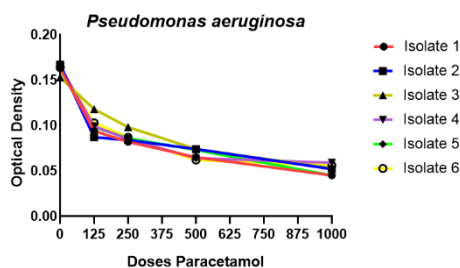


Figure 5.1 Graph of decreased optical density of biofilm *Pseudomonas aeruginosa* with exposure to paracetamol

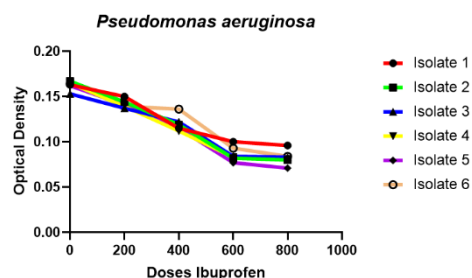


Figure 5.3 Graph of decreased optical density of biofilm *Pseudomonas aeruginosa* with exposure to ibuprofen

Inhibition of the formation of *P. aeruginosa* biofilm

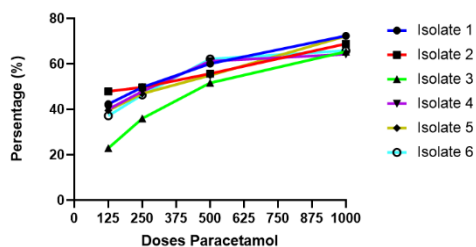


Figure 5.2 Graph of percentage resistance of biofilm *Pseudomonas aeruginosa* with exposure to paracetamol

Inhibition of the formation of *P. aeruginosa* biofilm

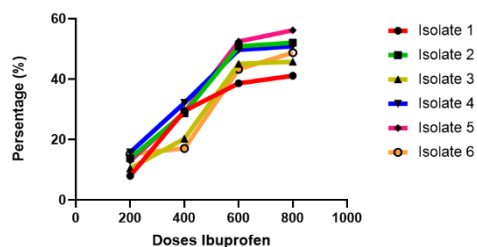


Figure 5.4 Graph of percentage resistance of biofilm *Pseudomonas aeruginosa* with exposure to ibuprofen

This in vitro study shows that paracetamol and ibuprofen have an effect as antibiofilms with concentration-dependent activity where the concentration / dose exposed is higher along with it providing a stronger effect to inhibit the formation of biofilms (Shah et al., 2018; Gerner et al., 2020; Paes Leme & da Silva, 2021). The inhibition of biofilm formation is seen by the reduction of biofilm biomass attached to the microtiter plate, characterized by a decrease in the optical density value measured by elisa reader. Based on preliminary test data, *biofilm-forming Pseudomonas aeruginosa* has an average OD value of 0.162 after the isolates were used in subsequent tests with the administration of paracetamol and ibuprofen exposure to a significant decrease in OD. This is closely related to the decrease in regulation or expression of components in the formation of biofilms. Biofilm expression is associated with genes involved in the process of attachment to the surface and the production of biofilm matrix components that are believed to play an important role in the formation of biofilms. Biofilm transcription profiles are

dominated by genes involved in stress response and adaptation to oxygen and iron (Thoming et al., 2020).

The work of paracetamol and ibuprofen in inhibiting the formation of biofilms by inhibiting several virulence factors regulated by genes contained in the Quorum sensing system which will further help the body's immune system to get rid of pathogens. Quorum sensing regulates many virulence factors of *Pseudomonas aeruginosa* adjusted to the density of the cells formed, including several secreted factors such as elastase, phospholipase C, lecithinase, rhamnolipids and through secondary metabolism such as pyocyanin (Köhler, Guanella, Carlet, & Van, 2010). From this study, it can also be seen that the inhibition of the formation of *pseudomonas aeruginosa* biofilm with ibuprofen a maximum of 49.13% at a dose of ibuprofen 800mg. Like paracetamol, the inhibitory effect of the formation of *P. aeruginosa* biofilm using ibuprofen has been seen from the smallest dose of exposure, namely at a dose of 200mg. Although from statistical tests there are insignificant differences in the dosage of 600mg and 800mg. So it can be said that the dosage of 600mg and 800mg has an effect that is not much different (Dai et al., 2019).

The results of statistical tests comparing doses capable of inhibiting, paracetamol and ibuprofen showed significant differences for all doses, except ibuprofen doses of 600mg and 800mg were not obtained significantly different from  $p=0.422$  values, can be seen in Tables 5.3 and 5.4.

Table 5.3  
Significance between paracetamol doses from Post-Hoc test results using Least Significant Difference

Test Doses	Least Significant Difference			
	Dose 125mg	Dose 250mg	Dose 500mg	Dose 1000mg
Dose 125mg	-	0.007	0.000	0.000
Dose 250mg	0.007	-	0.000	0.000
Dose 500mg	0.000	0.000	-	0.001
Dose1000mg	0.000	0.000	0.001	-

Table 5.4  
Significance between doses of ibuprofen from mann Whitney's Post-Hoc test results

Test Doses	Mann Whitney			
	Dose 200mg	Dose 400mg	Dose 600mg	Dose 800mg
Dose 200mg	-	0.004	0.004	0.004
Dose 400mg	0.004	-	0.004	0.004
Dose 600mg	0.004	0.004	-	0.422
Dose 800mg	0.004	0.004	0.422	-

Clinical meaning of this study, paracetamol and ibuprofen can be used to inhibit (bacteriostatic) the formation of biofilm-producing *biofilm Pseudomonas aeruginosa* bacteria. Adjusted to the recommended dosage of use guidelines. Because from the results of the study, the smallest exposure dose of 125mg for paracetamol and 200mg for ibuprofen has shown inhibitory power, although it is still minimal compared to the highest exposure dose in this study. (Dai et al., 2019)

## Conclusion

Paracetamol has the inhibitory power of biofilm formation of clinical isolates stored the best *Pseudomonas aeruginosa* bacteria at a dose of paracetamol 1000mg compared to doses of 125mg, 250mg and 500mg and in Ibuprofen has

the inhibition of biofilm formation of clinical isolates stored *bacteria Pseudomonas aeruginosa* best at doses of ibuprofen 800mg compared to doses of 200mg, 400mg and 600mg in invitro assays. Statistically there was a significant difference in the decrease in Optical density of the biofilm *Pseudomonas aeruginosa* with exposure to doses of paracetamol and ibuprofen, except at doses of 600mg and 800mg of ibuprofen.

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### References

- Balouiri, M., Sadiki, M., & Ibsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, 6(2), 71-79.
- Dai, L., Wu, T. Q., Xiong, Y. S., Ni, H. B., Ding, Y., Zhang, W. C., ... & Yu, J. (2019). 'Ibuprofen-mediated potential inhibition of biofilm development and quorum sensing in *Pseudomonas aeruginosa*'. *Life sciences*, 237, 116947.
- Gerner, E., Almqvist, S., Werthén, M., & Trobos, M. (2020). Sodium salicylate interferes with quorum-sensing-regulated virulence in chronic wound isolates of *Pseudomonas aeruginosa* in simulated wound fluid. *Journal of medical microbiology*, 69(5), 767.
- Hall, S., McDermott, C., Anoopkumar-Dukie, S., McFarland, A. J., Forbes, A., Perkins, A. V., ... & Grant, G. D. (2016). 'Cellular effects of pyocyanin, a secreted virulence factor of *Pseudomonas aeruginosa*'. *Toxins*, 8(8), 236.
- Haney, E. F., Trimble, M. J., & Hancock, R. E. (2021). 'Microtiter plate assays to assess antibiofilm activity against bacteria'. *Nature Protocols*, 16(5), 2615-2632.
- Haney, E. F., Trimble, M. J., Cheng, J. T., Vallé, Q., & Hancock, R. E. (2018). 'Critical assessment of methods to quantify biofilm growth and evaluate antibiofilm activity of host defence peptides'. *Biomolecules*, 8(2), 29.
- Hassan, A., Usman, J., Kaleem, F., Omair, M., Khalid, A., & Iqbal, M. (2011). Evaluation of different detection methods of biofilm formation in the clinical isolates. *Brazilian journal of infectious diseases*, 15, 305-311.
- Jani, J. R., Bajamal, A. H., Utomo, S. A., Parenrengi, M. A., Fauzi, A. A., Utomo, B., & Dwihapsari, Y. (2021). Correlation between magnetic resonance imaging (MRI) and dynamic mechanical analysis (DMA) in assessing consistency of brain tumor. *International Journal of Health & Medical Sciences*, 4(2), 260-266. <https://doi.org/10.31295/ijhms.v4n2.1737>
- Köhler, T., Guanella, R., Carlet, J., & Van Delden, C. (2010). 'Quorum sensing-dependent virulence during *Pseudomonas aeruginosa* colonisation and pneumonia in mechanically ventilated patients'. *Thorax*, 65(8), 703-710.
- Paes Leme, R. C., & da Silva, R. B. (2021). 'Antimicrobial Activity of Nonsteroidal Anti-inflammatory Drugs on Biofilm: Current Evidence and Potential for Drug Repurposing'. *Frontiers in microbiology*, 2115.

- Pinto, H., Simões, M., & Borges, A. (2021). 'Prevalence and impact of biofilms on bloodstream and urinary tract infections: A systematic review and metaanalysis'. *Antibiotics*, 10(7), 825
- Seleem, N. M., Atallah, H., Abd El Latif, H. K., Shaldam, M. A., & El-Ganiny, A. M. (2021). 'Could the analgesic drugs, paracetamol and indomethacin, function as quorum sensing inhibitors?'. *Microbial Pathogenesis*, 158, 105097.
- Shah, P. N., Marshall-Batty, K. R., Smolen, J. A., Tagaev, J. A., Chen, Q., Rodesney, C. A., ... & Cannon, C. L. (2018). 'Antimicrobial activity of ibuprofen against cystic fibrosis-associated gram-negative pathogens'. *Antimicrobial agents and chemotherapy*, 62(3), e01574-17.
- Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. *International Journal of Health Sciences*, 5(3), x-xv. <https://doi.org/10.53730/ijhs.v5n3.2938>
- Thöming, J. G., Tomasch, J., Preusse, M., Koska, M., Grahl, N., Pohl, S., ... & Häussler, S. (2020). 'Parallel evolutionary paths to produce more than one *Pseudomonas aeruginosa* biofilm phenotype'. *npj Biofilms and Microbiomes*, 6(1), 1-13
- Zimmermann, P., & Curtis, N. (2017). 'Antimicrobial effects of antipyretics'. *Antimicrobial agents and chemotherapy*, 61(4), e02268-16.