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The effect of prolonged exposure to the antibiotic meropenem on the resistance characteristics of *Acinetobacter baumannii* in vitro

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ABSTRACT

Introduction: To prove the effect of prolonged exposure to the antibiotic meropenem on the emergence of Acinetobacter baumannii resistance in vitro.

Methods: Meropenem-susceptible Acinetobacter baumannii isolates were exposed to 0.1 mg/ml meropenem and incubated for 8 days. The optical density and number of Acinetobacter baumannii bacterial colonies were observed every 24 hours. The average of optical density and the number of *Acinetobacter baumannii* bacterial colonies growing every 24 hours were analyzed using the Kruskal-Wallis test.

Result: The results showed a decrease in the average optical density value and the number of Acinetobacter baumannii bacterial colonies from day 1 to day 6 of incubation. However, there was an increase in the average optical density and the number of *Acinetobacter baumannii* bacterial colonies starting on day 7 and day 8.

Conclusion: Exposure to the antibiotic meropenem 0.1 mg/ml for 6 days might be associated with the emergence of Acinetobacter baumannii resistance in vitro.

Keywords: *Acinetobacter baumannii*, antimicrobial resistance, meropenem.
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INTRODUCTION

The prevalence of multidrug-resistant organisms (MDRO) infections continues to rise and has become a particular concern. According to the WHO, the carbapenem-resistant *Acinetobacter baumannii* (CRAB) is a critical priority pathogen for which new therapeutic agents are urgently needed.[1] The Global Antimicrobial Resistance and Use Surveillance System (GLASS) in 2022 reported that the prevalence of carbapenem resistant *Acinetobacter spp* was more than 56% [2], in addition the Surveillance for Indonesian Network on Antibiotic Resistance (SINAR) in 2021 found that the prevalence of CRAB in the intensive care unit (ICU) was more than 70%.[3] The clinical implications of meropenem resistance are extensive. Patients infected with meropenem-resistant strains frequently face delayed and insufficient treatment, leading to increased mortality rates and longer hospitalizations. Strategies to address meropenem resistance include implementing antibiotic stewardship programs, strengthening infection control measures, and developing new antimicrobial agents.[4]

Resistance to carbapenem antibiotics occurs through both vertical and horizontal resistance mechanisms, including the presence of carbapenemase enzymes, target site modifications, porin alterations, and efflux pumps.[5] The carbapenemase enzymes, such as OXA and metallo-ß-lactamases, hydrolyze drugs, causing carbapenem inactivation and are easily transferred into the bacteria.[6–8] The presence of porin channels and other outer membrane proteins helps deliver drugs to target proteins. However, in carbapenem-resistant *Acinetobacter baumannii*, the porin channels are smaller, preventing the entry of drug molecules. Apart from that, changes in genes that code for protein targets and increased cellular functions involved in the production of efflux pumps play a role in the occurrence of resistance.[8] Antibiotic exposure is considered a key factor influencing the emergence and spread of antibiotic resistance, such that antibiotic-sensitive bacteria die. In contrast, resistant bacteria survive, a phenomenon known as selective pressure.[9] Identifying the duration of antibiotic resistance is important for antibiotic therapy reference. Some existing studies have shown that antibiotic resistance can develop rapidly, within 3 to 4 days; for example, bacteria such as *Enterobacter, Citrobacter*, and *Serratia spp*. can become resistant to third-generation cephalosporins, *Pseudomonas aeruginosa* to all antibiotics, and Staphylococcus to fluoroquinolones.[10] This study aimed to analyze the effect of prolonged exposure to the antibiotic meropenem on the emergence of meropenem resistance in *Acinetobacter baumannii* in vitro.

METHODS

Design

This study is an experimental laboratory study designed to observe the effect of prolonged exposure to the antibiotic meropenem at a dose of 0.1 mg/mL on

the emergence of antibiotic resistance in *Acinetobacter baumannii* bacteria in vitro prospectively. The experiment was conducted from February to August 2024. The treatment group was defined as the meropenem susceptible *Acinetobacter baumannii* isolate exposed to the antibiotic meropenem 0.1 mg/mL for 8 days. The control group was defined as the meropenem susceptible *Acinetobacter baumannii* isolate without antibiotic meropenem exposure for 8 days. There were 4 replications performed for each treatment group or control group. The bacterial growth was measured directly based on the colony count and indirectly using spectrophotometry, each 24 hours for 8 days for both groups. This study was approved by the medical ethics committee of the Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia (number 100/EC/KEPK/05/2023).

Bacterial isolate

The *Acinetobacter baumannii* isolate used in this study was susceptible to meropenem based on the Vitek 2 system. The isolate was the collection of bacterial stock in the Department of Clinical Microbiology, Dr. Saiful Anwar Hospital, Malang, Indonesia.

Antibiotic exposure

The 2.5 mL of 10^{10} CFU of *Acinetobacter baumanni* suspension in the Mueller Hinton broth media was inoculated into the 2.5 mL of 0.1 mg/mL of meropenem suspension and assigned as the treatment group. Both the treatment and control groups (without meropenem exposure) were replicated four times and incubated at 37 °C for eight days.

Bacterial growth measurement

Every 24 hours, ten microliters of bacterial suspension from both the treatment group and control group were inoculated onto MacConkey agar. The number of colonies was counted after incubation at 37 °C for 18-24 hours. In addition, the optical density of bacterial suspension in both the treatment group and control group was measured using spectrophotometry.

Statistical analysis

Data on the optical density and the number of bacterial colonies were analyzed using SPSS Statistics version 22.

Table 1. The optical density and colony count of *Acinetobacter baumannii* exposed to meropenem and no exposed

Day	Exposure to meropenem (treatment group)		No exposure to meropenem (control group)	
	Average of the optical density ± SD	Average of the colony count ± SD (CFU) x10^6	Average of the optical density ± SD	Average of the colony count ± SD (CFU) x 10^6
0	1.000	10,000	1.000	10,000
1	0.593 ± 0.074	174,6 ± 156	0.910 ± 0.133	22,495.3 ± 4.470
2	0.569 ± 0.089	106,3 ± 107	0.987 ± 0.140	19,985.0 ± 6.863
3	0.483 ± 0.083	113,3 ± 153	0.993 ± 0.248	166,215.6 ± 96.618
4	0.485 ± 0.092	100,3 ± 86	0.980 ± 0.275	30,745.3 ± 22.211
5	0.460 ± 0.084	63,2 ± 87	0.986 ± 0.299	26,710.6 ± 7.040
6	0.438 ± 0.070	21,4 ± 8	0.979 ± 0.424	25,167.5 ± 7.927
7	0.549 ± 0.112	224,0 ± 103	1.022 ± 0.369	21,657.1 ± 7.771
8	0.628 ± 0.153	2,075.1 ± 1,333	1.029 ± 0.44	20,006.8 ± 6.272
p	< 0.001	<0.001	0.983	<0.001

SD=standard of deviation; CFU=colony forming units

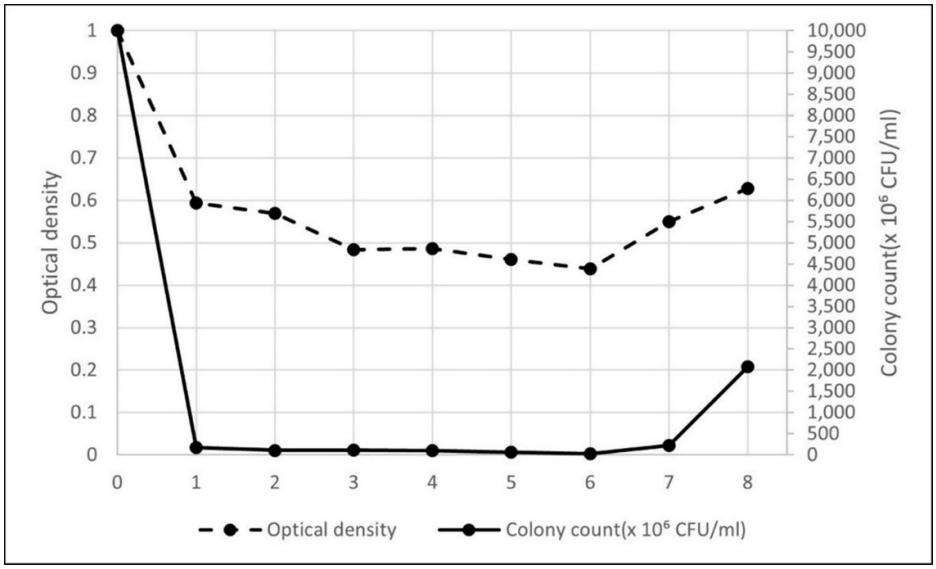

Figure 1. The average of optical density and the number of *Acinetobacter baumannii* colonies after being exposed to meropenem 0.1 mg/mL for 8 days.

RESULTS

Table 1 and Figure 1 show the decrease in optical density and the number of colonies after being exposed to the meropenem 0.1 mg/ml until day 6, but then increase on day 7 and day 8. The statistical analysis showed a significant decrease in the optical density and the number of colonies (*p* value <0.001 and <0.001, respectively).

DISCUSSION

This study found that exposure to 0.1 mg/mL of meropenem for 6 days had induced resistance in *Acinetobacter baumannii* bacteria, which were initially sensitive to meropenem. The measurements of bacterial growth directly through colony counting aligned with optical density at each observation period. The decrease in optical density and colony count from day 1 to day 6, followed by increasing on day 7, indicated the occurrence of selective pressure on the *Acinetobacter baumannii* due to meropenem exposure. Selective pressure arises from bacterial exposure to an antibiotic, leading bacteria to adapt

through mutations that introduce new genes.[11] This finding is consistent with the previous study conducted by Balazs et al., which reported that meropenem-resistant bacteria replicated, resulting in increased numbers of resistant bacteria.[12] Similar findings were reported by Rivani et al., where exposure to a combination antibiotic regimen, including meropenem, led to an increase in bacterial colonies and MIC values.[13] An animal study conducted in California in 2019 on Webster mice infected with *Acinetobacter baumannii*, and treated systemically with increasing doses of meropenem, showed an increase in the number of bacteria isolated from mouse tissues, demonstrating that *Acinetobacter baumannii* infections in Webster mice became resistant to meropenem.[14]

The mechanisms of resistance in *Acinetobacter baumannii* to meropenem include increased production of beta-lactamase enzymes, including carbapenemases like OXA or NDM-1, enhanced porin secretion reducing meropenem permeability, increased expression or activity of efflux pumps expelling antibiotics from the bacterial cell, and potential mutations in antibiotic target sites such as penicillin-binding proteins (PBP).[15] Nguyen and Joshi's review article mentioned that *Acinetobacter baumannii* resistance can involve the development of efflux pumps that enhance the expulsion of antibiotics from the cell before reaching effective concentrations. Other resistance mechanisms include changes in bacterial cell membrane structure, reducing antibiotic permeability, and genetic regulatory mechanisms controlling gene expression in response to selective pressure from meropenem. The resistance gene encoding for meropenem is *OXA*.[16] Similar results were obtained in Balazs et al.'s study, where changes in the *oxa* gene resulted in new mutations.[12] Previous research has shown that repeated antibiotic exposure can induce mutations leading to antibiotic resistance, allowing bacteria to survive and proliferate by evading the effects of meropenem.[17]

The implications of this study highlight the importance of monitoring the duration of antibiotic use in patients with bacterial infections, particularly with meropenem for treating *Acinetobacter baumannii* infections. Prolonged use of meropenem beyond seven days for *Acinetobacter baumannii* infections may lead to the emergence of carbapenem-resistant *Acinetobacter baumannii* strains. This condition also occurs in Pseudomonas aeruginosa bacteria, which will become resistant after 8 days of exposure to meropenem, based on research by Yusuf et al.[18] This study underscores the need for the development of new and innovative antimicrobial strategies. Developing new antibiotics with different mechanisms of action or structural modifications may be key to addressing the increasing challenge of bacterial resistance. Combination therapy approaches and the use of adjuvants could also be promising solutions to enhance antibiotic effectiveness and slow the development of bacterial resistance.

This study has some limitations. First, the minimum inhibition concentration of the *Acinetobacter baumannii* isolate after meropenem exposure was not measured; therefore, the status of meropenem resistant of the isolate was still unclear. Second, the carbapenem-resistant genes were not identified in this study; thus resistance mechanisms of the *Acinetobacter baumannii* due to meropenem exposure for 6 days were unknown. Third, this study was conducted in vitro, which might generate different respond in the in vivo study. Further investigation is needed to improve the quality of the study by measuring the minimum inhibition concentration of the isolate and identify the resistance genes after antibiotic exposure.

CONCLUSION

The exposure to 0.1 mg/ml meropenem for 6 days lead to the development of resistance in *Acinetobacter baumannii* isolates in vitro.

CONFLICT OF INTEREST

No conflict of interest in this study.

FUNDING

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AUTHORS CONTRIBUTION

Conceptualization, S.I.W.,D.S.,and Y.I.S; methodology, Y.I.S. and D.S.; software, Y.I.S and D.S.; validation, D.S and S.I.W.; formal analysis, D.S. and S.I.W.; investigation, Y.I.S., D.S. and S.I.W.; resources, Y.I.S. and D.S.; data curation, Y.I.S and D.S.; writing—original draft preparation, Y.I.S. and D.S.; writing—review and editing, Y.I.S, D.S. and S.I.W.; supervision, S.I.W. and D.S. All authors have read and agreed to the published version of the manuscript.

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