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Progressivity of in vitro resistance of *Klebsiella pneumoniae* ESBL to meropenem and amikacin therapy in specimens from the intensive care unit of Saiful Anwar Hospital, Malang

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ABSTRACT

Introduction: Multidrug-resistant organism (MDRO) infections pose a significant global health challenge. The WHO has identified *Klebsiella pneumoniae* as a high-priority pathogen responsible for such infections. The presence of K. *pneumoniae* ESBL can lead to higher mortality rates. Currently, antibiotics like meropenem and amikacin are used to treat K. *pneumoniae* ESBL infections. However, there is no existing research on how antibiotic resistance develops over time. This study aimed to investigate the progression of *in vitro resistance* of K. *pneumoniae* ESBL to meropenem and amikacin in samples from the ICU at Saiful Anwar Hospital.

Methods: This study uses the AZDZST method, a novel technique called the Ameri-Ziaei double antibiotic synergism test for assessing antimicrobial synergy. It functions as a disk diffusion test designed for double antibiotic synergy, with inhibition zone diameters providing data similar to other disk diffusion methods. This makes AZDAST simple to perform using the Kirby-Bauer disk susceptibility test. The method is independent and does not require reference tables like CLSI guidelines. Results are interpreted by comparing inhibition zone sizes around single and dual disks; a reduction in zone size indicates resistance. Klebsiella pneumoniae ESBL isolates, confirmed by Vitek 2, were tested for susceptibility to meropenem and amikacin. These isolates were exposed to 10 µg meropenem disks, 30 µg amikacin disks, and a combination of both disks using AZDAST over 14 days. The inhibition zones were measured after 18-24 hours of incubation at 37°C.

Results: Amikacin antibiotics exhibited resistance by the fifth day of exposure, while meropenem showed resistance on the 12th day across three samples, and on the 14th day in one sample. The combination of amikacin and meropenem also exhibited resistance by day 14 of exposure. There was no significant difference in the duration of resistance between monotherapy and the combination therapy of meropenem and amikacin.

Conclusion: Meropenem shows resistance by day 12, while amikacin is resistant from day 5. There is no significant difference in resistance duration between monotherapy and combination antibiotic treatments against Klebsiella pneumoniae ESBL in vitro.

Keywords: amikacin, antimicrobial resistance, *Klebsiella pneumoniae* ESBL, meropenem.
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INTRODUCTION

Klebsiella pneumonia ESBL is a strain of Klebsiella pneumonia that produces the enzyme Extended Spectrum Beta Lactamase (ESBL). This enzyme can hydrolyze or inactivate antibiotics like aztreonam, broad-spectrum cephalosporins, and broad-spectrum penicillins. However, ESBL enzymes do not affect carbapenems and generally do not hydrolyze cephamycins such as cefoxitin and cefotetan. The activity of ESBLs can be blocked by beta-lactamase inhibitors like clavulanic acid.[1] The difference between ESBL-producing and carbapenemase-producing Klebsiella pneumoniae lies in their antibiotic resistance levels, especially regarding susceptibility to carbapenems. A key resistance mechanism in gram-negative bacteria is the acquisition of carbapenemases—enzymes that break down these antibiotics.[2]

According to the CDC, in 2019, Multidrug-Resistant Organism (MDRO) infections were a global health threat, causing at least 2.6 million deaths, and ESBL has increased by 50% from the previous year.[3] WHO established a list of "priority status" pathogens, with *Klebsiella pneumoniae* as one of the pathogens causing infection.[4] *Klebsiella pneumoniae ESBL* infections can increase morbidity and mortality, require extended hospital stays, and ultimately cost more.[3] In patients with alcoholism and septicemia, mortality ranges from 50% to 100%.[5] In Southeast Asia (SEA), the prevalence of ESBL K. pneumoniae in SEA is estimated to be 27% (CI 32–100) in 2023.[6] The prevalence of patients identified with positive ESBL-producing Klebsiella pneumoniae was 80.95% in the ICU room of PKU

Muhammadiyah Hospital, Yogyakarta, from January 2023 to December 2023.[7] Meropenem and Amikacin antibiotics are used as therapies for *Klebsiella pneumoniae ESBL* infections, but until now, there has been no research on the progressiveness of antibiotic resistance. This research aims to study the progressivity of in vitro resistance of *Klebsiella pneumoniae* ESBL to meropenem and amikacin therapy in specimens from the Intensive Care Unit of Saiful Anwar Hospital, Malang.

METHODS

Design, Time, and Place of Research

This research is a cross-sectional experiment that observes meropenem and amikacin during the emergence time of antimicrobial resistance in vitro using the AZDAST method. The population in this study was a *Klebsiella pneumoniae ESBL* bacterial isolate from the Regular ICU room and examined at the Clinical Microbiology Laboratory of Dr. Saiful Anwar Malang Hospital in March-April 2024.

Sampling Technique

The determination of this study sample used random purposive sampling on bacterial species according to the semi-automatic machine Vitex 2 Automated Microbiology System, which showed *Klebsiella Pneumoniae ESBL* isolates from patient specimens admitted to the ICU room of RSUD Dr. Saiful Anwar Malang. Inclusion criteria were *Klebsiella pneumoniae ESBL* culture from patient specimens taken only once and treated in the ICU room of RSUD Dr. Saiful Anwar Malang in March-April 2024 with identical antibiotic profiles. The exclusion criteria were *Klebsiella pneumoniae ESBL,* which showed resistant or intermediate results to amikacin and meropenem. The number of samples was determined using the experimental formula, and four specimens were obtained.

Diffusion Agar Disk with AZDAST Method

This new method for evaluating antimicrobial synergy is based on the Kirby-Bauer principle (**Figure 1**). One of the advantages of the AZDAST test compared to the Kirby-Bauer method is that AZDAST can be used to assess the synergy of combinations of two antibiotics. The diameter of the zones of inhibition for bacterial growth in the AZDAST method is measured in millimeters, similar to other disk diffusion methods. In the AZDAST method, two antibiotic disks are stacked on a Muller Hinton Agar.[8]

The following steps should be carried out to run AZDAST, in the same order shown in the graphical abstract.

1. Provide a sterile glass petri dish with a diameter of 12cm (see **Figure 1**, step 1). Note: You may choose different sizes or disposable petri dishes for AZDAST, but ensure compliance with regulations such as agar depth.
2. Create an adhesive by mixing a glue that contains 1.5 times the concentration of molten, cooled (44–48°C) autoclaved Mueller-Hinton agar. Apply this adhesive to the bottom of the petri dish to affix the antibiotic paper disk. To prevent the agar from solidifying too quickly, consider keeping it on a heater while working.
3. Dip the first antibiotic paper disk in the glue (i.e. "A" disk in the **Figure 1** step 2).
4. Place the smeary "A" disk on the designated spot on the petri dish floor (**Figure 1**: steps 3 and 4). Note: The graphical abstract only illustrates the combination site of the dish. All positions in the following pattern should be placed before pouring the agar (referred to as AZDAST petri dish).

GRAPHICAL ABSTRACT

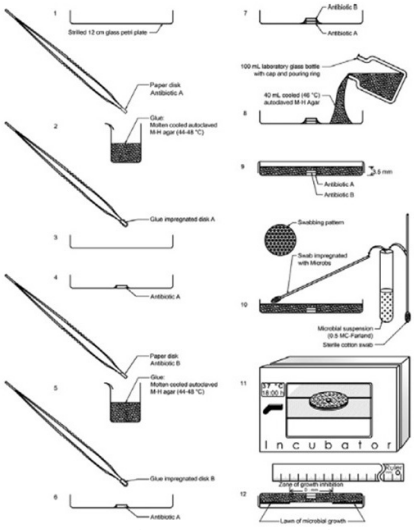

Figure 1. Schematic steps of AZDAST method

5. Similarly, the second disk ('B') was smeared and affixed in its designated positions (**Figure 1**: steps 5, 6, and 7).
6. The Petri dish is now filled with 40 mL of lukewarm autoclaved Mueller-Hinton medium (see **Figure 1**: step 8). It is heated in a 46°C shaking water bath for 30 minutes. The agar should be approximately 3.5 mm deep, though 3 to 4 mm is acceptable (specific details are not provided in this paper). Detecting synergism among multiple antibiotics can be done using several plates or by performing tests on separate plates arranged as a combination, cummulative, or deep Kirby-Bauer plates, with a simpler disk placement. Nonetheless, all plates must have an equal agar depth.
7. Allow the agar to solidify within a few minutes. The AZDAST petri plate is now prepared for inoculation (see **Figure 1**: step 9).
8. The inoculum was prepared using direct colony suspensions from a fresh 24-hour culture, calibrated to a 0.5 McFarland standard. This study achieved an optical density of 0.08–0.10 at 625nm.
9. The plate was inoculated with a sterile swab following the spread plate method (see **Figure 1**: step 10).
10. Incubate the plate at 37°C for 18 hours (see **Figure 1**: step 11). Note: The rule of 15, 15, and 15 should be followed and can be applied.
11. Similarly to the Kirby-Bauer method (CLSI guideline), the zone of inhibition's diagonal can be measured using a ruler or calliper (see **Figure 1**: step 12).
12. Use a ruler with appropriate units to measure the diameter of the inhibition zone mm.[8]

In this study, the method begins by adapting the AZDAST method, which will be used in 4 groups. This method begins with preparing Mueller-Hinton solid media according to the manufacturer's instructions—separate 10 ml in a separate tube before autoclaving. Perform sterilization using an autoclave at 121˚C for 15 minutes. Prepare the antibiotic paper disks (Amikacin disk 30 µg, Meropenem disc 10 µg). Petri dish for single disk antibiotic Amikacin 30 µg, Meropenem

disk 10 µg, with a separate plate. Petri dish for double disk antibiotics, Amikacin disk 30 µg + Meropenem disk 10 µg (2 paper disks antibiotics stacked into one) with its plate. Attach the antibiotic to the bottom of the inner petri dish using sterilized, still-liquid Mueller-Hinton agar, ensuring it stays in place when poured. Once the agar solidifies, pour enough into the dish to fill half its height and allow it to solidify. To prepare Klebsiella pneumoniae ESBL bacteria, pick several colonies from solid media, place them into a test tube with sterile 0.9% NaCl, homogenize the sample, and perform a turbidity test with a spectrophotometer or nephelometer to achieve a 0.5 McFarland standard.

Use a cotton swab to inoculate the Klebsiella pneumoniae ESBL bacterial suspension from each specimen and evenly spread it over the entire agar surface, which is divided into four treatment groups: control, amikacin, meropenem, and amikacin-meropenem combination. Incubate the plates upside down at 37 °C for 18-24 hours. After 18-24 hours, measure the diameter of the inhibition zone with a ruler with units of mm and take isolates around the inhibition zone to be re-inoculated with the AZDAST method. To ensure that the colony taken is pure, colonies that have the same shape and color at the edge of the inhibition zone were selected. The edge was chosen because it represents growth under the highest tolerated antibiotic concentration. Clusters or confluent regions. Repeat the cycle until resistant, with a maximum of 14 days. If the inhibition zone has reached resistance, i.e., amikacin ≤16 mm, meropenem ≤19 mm, stop until this stage. The resistance zone on amikacin and meropenem combination plates follows the smallest zone of ≤16 mm.[9]

Statistical Data Analysis

The resulting data will be analysed using *SPSS*, followed by the normality and homogeneity tests. Then, proceed with the comparison test and correlation test.

RESULTS

There are four samples, consisting of 3 samples of blood specimens (samples 2, 3, 4) and one sample of FOB specimens (sample 1) obtained from specimens of patients admitted to the ICU room of Dr. Saiful Anwar Malang Hospital in March-April 2024. The antibiotic sensitivity profiles of the four specimens were identical, with one difference in ceftazidime antibiotics. Table 1 shows bacterial characteristics of the research samples.

Inhibition Zone Diameter of the Control Group

All control groups exhibited growth across the entire Mueller-Hinton agar (MHA) media surface, with no zones of inhibition observed throughout the 14-day treatment period (Table 2 and Figure 2).

Inhibition Zone Diameter of Amikacin Group

The results of the Amikacin group showed that on the fifth day, the four specimens tested had reached a resistant diameter of ≤16 millimetres (Table 3 and Figure 3).

Table 1. Antibiotic sensitivity characteristics of research data

	Sample 1	Sample 2	Sample 3	Sample 4
Ampicillin	R	R	R	R
Ampicillin Sulbactam	R	R	R	R
Peperacillin Tazobactam	R	R	R	R
Cefazoline	R	R	R	R
Ceftazidime	S	R	S	R
Ceftriaxone	R	R	R	R
Cefepime	S	S	S	S
Ertapenem	S	S	S	S
Meropenem	S	S	S	S
Amikacin	S	S	S	S
Gentamicin	R	R	R	R
Ciprofloxacin	R	R	R	R
Tigecycline	S	S	S	S
Trimethoprim/Sulfamethoxazole	R	R	R	R

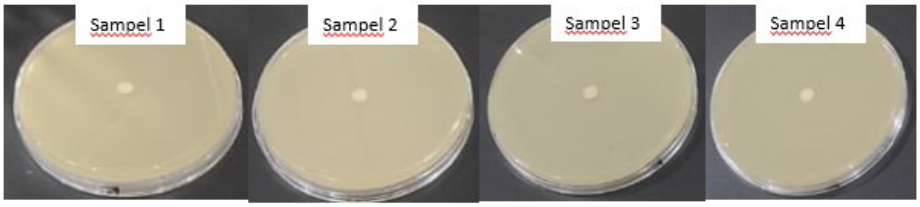

Figure 2. Control group inhibition zone.

Table 2. Control group inhibition zone diameter

	Inhibition Zone (mm)													
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Sample 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sample 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sample 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sample 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-

The Shapiro-Wilk normality test results indicated that the inhibition zone diameter from disk diffusion across 4 samples over 5 days was generally normally distributed (p> 0.05), confirming the assumption of normality. The Levene homogeneity test also showed p> 0.05, indicating that the homogeneity of variances was satisfied. Subsequently, the ANOVA test revealed p < 0.05, suggesting a significant difference in inhibition zones based on the number of antibiotic exposure days. The Pearson correlation test yielded a p-value < 0.05, demonstrating a significant correlation between the study days and inhibition zone size. Additionally, the regression analysis showed a significance value of p < 0.05, confirming that the number of exposure days influences the inhibition zone.

Inhibition Zone Diameter of Meropenem Group

The results of the Meropenem group showed that on day 12, specimens 1, 2, and 4 were resistant, while specimen number 3 was resistant on day 14 (resistant diameter ≤ 19 millimetres; Table 4 and Figure 4). The Shapiro-Wilk normality test indicates that all samples are normally distributed (p > 0.05), meeting the normality assumption. The Levene homogeneity test results show p > 0.05, confirming that the homogeneity criteria are satisfied. The ANOVA test revealed p < 0.05, indicating a significant difference in the inhibition zone across different antibiotic exposure days. The Pearson correlation analysis also showed p < 0.05, demonstrating a significant relationship between the study day and the inhibition zone size. Additionally, the regression analysis produced a p-value < 0.05, confirming that the number of exposure days significantly impacts the inhibition zone.

Table 3. Inhibition zone diameter of amikacin group

	Inhibition Zone (mm)				
	Day 1	Day 2	Day 3	Day 4	Day 5
Sample 1	28	27	25	23	16
Sample 2	27	26	23	22	16
Sample 3	30	29	25	23	12
Sample 4	27	25	25	23	11

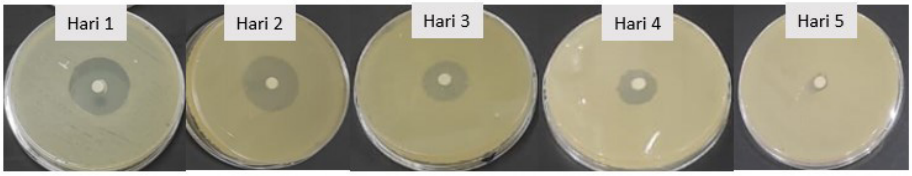

Figure 3. Amikacin group inhibition zone

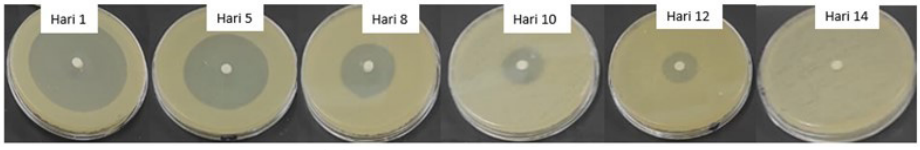

Figure 4. Meropenem group inhibition zone

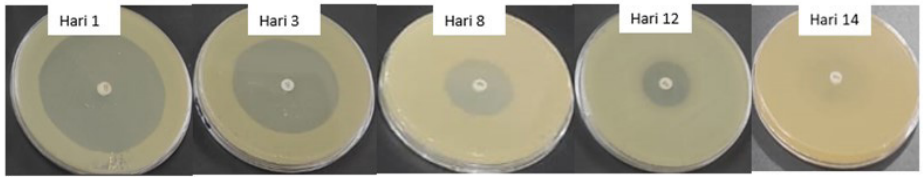

Figure 5. Meropenem amikacin combination group inhibition zone

Table 4. Meropenem group inhibition zone diameter

	Inhibition Zone (mm)													
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Sample 1	54	63	52	60	32	50	35	73	50	33	34	14	-	-
Sample 2	51	63	51	63	30	50	33	63	50	24	22	10	-	-
Sample 3	52	32	52	63	33	42	33	53	40	33	50	45	30	-
Sample 4	48	63	48	59	31	49	60	48	40	24	36	13	-	-

Table 5. Inhibition zone diameter group meropenem amikacin combination

	Inhibition Zone (mm)													
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Sample 1	54	63	52	63	32	52	33	55	55	25	38	28	24	-
Sample 2	52	63	52	60	30	52	33	52	50	25	24	23	18	-
Sample 3	52	63	52	63	30	55	33	52	60	33	53	34	40	-
Sample 4	48	60	49	58	32	50	59	48	55	24	40	20	24	-

Meropenem Amikacin Combination Group Inhibition Zone Diameter

The Meropenem Amikacin combination group *results* showed that four specimens on day 14 were resistant (resistant diameter ≤16 millimetres; **Table 5** and **Figure 5**).

The Shapiro-Wilk normality test indicates all samples are normally distributed ($p > 0.05$), confirming normality. The Levene homogeneity test also shows $p > 0.05$, confirming the homogeneity criteria are met. The ANOVA test reveals $p < 0.05$, indicating a significant difference in the inhibition zone across different antibiotic exposure days. The Pearson correlation test yields a p-value < 0.05, showing a significant relationship between the study day and the inhibition zone. Finally, the regression analysis confirms this with a p-value < 0.05, suggesting that the number of exposure days influences the size of the inhibition zone.

DISCUSSIONS

Advantages of the new method include (1) AZDAST is a qualitative, numerically scaled approach that involves comparing inhibition zone diameters in millimeters; (2) It can be conducted before more time-consuming quantitative tests, allowing for initial selection of the most potent synergistic combination, which can later be quantified through other methods; (3) AZDAST functions as an independent interpretive test that does not require standard tables; (4) The method uses routinely available laboratory materials; (5) Its procedure and interpretation are straightforward and easily understood, making it accessible beyond specialized research or reference labs.

The limitation of this method is that the area of the inhibition zone is influenced by the size of the disc, and the intensity of antibiotic interaction is influenced by the interaction of the concentrations of the two antibiotics, thus affecting the inhibition zone. Using antibiotic discs with comparable concentrations from the same brand and the same diameter can be used to mitigate these shortcomings. The depth of the agar affects the diameter of the inhibition zone.

The thing that might affect the results is the further distance of the deeper plate from the surface, so that there will be an obstacle to diffusion to the surface where the bacteria are inoculated. This possibility has been tested using two plates: a) an antibiotic disk at the bottom of the plate and an empty disk, b) an empty disk at the bottom of the plate and an antibiotic disk above. From the results of the experiment, no evidence was found of any significant difference between the two locations.[8]

The number of samples that met the inclusion and exclusion criteria in this study was four samples, consisting of 3 samples from blood specimens (sample 2,3,4) and one sample of a FOB specimen (sample 1). Based on research in Iran on the molecular, serotype, phenotypic, and genotypic characteristics of *Klebsiella pneumoniae* resistance isolated from different samples in hospitals showed the results that the determinants of antibiotic resistance are the presence of resistance genes owned by the bacteria. So, the difference in the samples received is not a determinant of antibiotic resistance.[10]

In **Table 1**, the antibiotic sensitivity characteristics of the study data showed an identical profile, with one difference in ceftazidime sensitivity. This difference could be due to different ESBL encoding genes among the four specimens. Based on the type of ESBL gene, CTX-M type, SFO type 1, and BES-1 have less ability to hydrolyse ceftazidime. Based on CLSI, the sensitive MIC for ceftazidime is ≤ 4 µg/mL, while the MIC of the two specimens is = 4 µg/mL; this indicates that the MIC is within the maximum sensitive limit, close to intermediate. Therefore, ceftazidime antibiotic should not be used as ESBL therapy because it is feared that it will become insensitive. Based on the history of antibiotic administration of the samples studied, it turned out to be very diverse, and the length of days of antibiotic administration was not known from the patient's medical record data; this would significantly affect the study results.

Inhibition Zone Results of Amikacin Antibiotics

In this study, four samples showed a zone of inhibition of resistant Amikacin antibiotics, reaching ≤ 16 mm on the fifth day. The minimum diameter on the fifth day was 11 mm. From the results of the regression test, the significance value is <0.0001 ($p < 0.05$), so it can be concluded that the length of exposure days affects the decrease in the inhibition zone of amikacin antibiotics. Continuous exposure to aminoglycoside antibiotics will cause *Klebsiella pneumoniae* to carry out resistance mechanisms in 5 ways as follows: enzymatic inactivation of antibiotics, changes in antibiotic targets, loss of porins, increased efflux pump antibiotic release, and biofilm formation.[11] The main method of resistance to the antibiotic Amikacin involves bacteria enzymatically modifying the drug using aminoglycoside-converting enzymes, which include acetyl-transferases, phosphotransferases, and nucleotidyltransferases. The plasmid-borne acetyltransferase is now identified as AAC (6)-Ib or AacA4. Previous research has found plasmid-mediated adenylyl transferases in strains of Klebsiella pneumoniae, Escherichia coli, Serratia marcescens, and Proteus vulgaris, all capable of using amikacin as a substrate. Additionally, resistance to amikacin by decreased drug uptake has been observed in K pneumoniae.[12] Although multiple resistance mechanisms to amikacin have been identified, the most common in clinical settings is acetylation at the 6-N position. This process is mediated by the enzyme AAC (6)-I, followed by a specific identifier, which typically results in resistance to aminoglycosides like amikacin, tobramycin, and kanamycin.[12]

Based on data on antibiotic sensitivity characteristics in four samples, this study shows that they resist gentamicin antibiotics. Amikacin and gentamicin belong to one class of aminoglycosides. Gentamicin was first isolated in 1966 from Micromonospora purpura, and after widespread use, it became clear that the main resistance mechanism was enzymatic modification by aminoglycoside-inactivating enzymes. These enzymes are categorized as acetyltransferases, phosphotransferases, and nucleotidyltransferases. In 1977, amikacin was developed as a semi-synthetic aminoglycoside to combat gentamicin resistance. It is produced by acylation with a γ-amino-α-hydroxybutyryl side chain on the C-1

amino group of the deoxytreptamine part of kanamycin A. The clinical resistance to amikacin primarily involves acetylation by the enzyme aminoglycoside 6-N-acetyltransferase type Ib [AAC(6)-Ib], which is encoded by genes located in introns, transposons, plasmids, and chromosomal DNA of Gram-negative bacteria.[12]

Based on the results of research in France, most gentamicin-resistant Enterobacteriaceae also have cross-resistance to tobramycin but are still susceptible to amikacin; this occurs because *Enterobacteriaceae* contain plasmid-mediated deactivating enzymes that are unable to react with amikacin. Resistance to amikacin tends to be relatively low (most have a Minimum Inhibitory Concentration (MIC) between 31 and 125 µg/ml); the decreased ability to accumulate intracellular amikacin appears to be the primary mechanism responsible for resistance, in addition to the acetylase (AAC (6')) process that inactivates amikacin. Organisms resistant to amikacin administration are usually also resistant to two other aminoglycoside antibiotics of the generation that came first.

Based on **Table 3**, the minor amikacin inhibition zone was obtained in sample 4 with a history of patients already getting meropenem antibiotics, which is by research in Los Angeles stating that the administration of meropenem in the last 30 days can increase the occurrence of amikacin resistance, it is just that until now it is still unknown the mechanism of how giving meropenem antibiotics can increase the incidence of amikacin resistance, so further research still needs to be done.[13]

Inhibition Zone Results of Meropenem Antibiotics

The resistance inhibition zone in three samples was achieved on day 12, while one sample showed a resistant zone on day 14. The emergence of *Klebsiella pneumoniae* resistance to meropenem antibiotics involves the mechanism of carbapenemase enzyme production.[13] The carbapenemase enzyme is caused by the production of β-lactamase, which can inactivate carbapenem and other β-lactam antibiotics.

In this study, for samples 1,2, and 4, based on the history of antibiotic administration to patients before specimen collection, they had received cefoperazone sulbactam, ceftriaxone, and meropenem therapy. β-lactam antibiotics, aminoglycosides, and quinolones are often used to treat *Klebsiella pneumoniae* infections. Through the mechanism of *chromosomal* and plasmid-encoded ARGs, which this pathogen possesses, it causes the spread of diverse resistance genes.[11] Therefore, samples 1,2 and 4 are more resistant to meropenem than sample 3, which did not get antibiotic exposure before specimen collection.

Based on this in vitro experiment's research, the regression test results showed a significance value of <0.0001 ($p < 0.05$). This indicates that the length of exposure days significantly affects the reduction in the meropenem antibiotic inhibition zone.

Inhibition Zone Results of Meropenem Amikacin Antibiotic Combination

In this study, all four samples exhibited a resistant inhibition zone on day 14 when using the combination of meropenem and amikacin, as shown in **Table 4**. The in vitro experiment results indicated a regression test significance value of <0.0001 ($p < 0.05$), demonstrating that the number of exposure days significantly affects the reduction in the inhibition zone of the meropenem and amikacin combination.

The effect of the meropenem amikacin combination based on the AZDAST method criteria showed no distinguishable results, where the combination showed the same inhibition zone as one of the single antibiotics.[14] This combination shows little difference in resistance time between the use of one type of antibiotic and the use of combined antibiotics. In this trend study, the use of amikacin within 5 days showed a resistant zone, while the meropenem group and the combination of amikacin and meropenem showed longer resistance. Amikacin resistance mainly occurs through acetylation at the 6-N position. The enzyme responsible, known as AAC (6)-I, along with its unique identifier, typically confers resistance to aminoglycosides.[12]

In the case of meropenem, resistance development is slower because carbapenems, along with other beta-lactams, are among the most potent options against a wide range of Gram-positive and Gram-negative bacteria. Its distinctive molecular structure includes a carbapenem combined with a beta-lactam ring, which grants it exceptional stability against most beta-lactamases, such as AmpC and ESBL. This high efficacy across many bacterial species and reduced susceptibility to common resistance mechanisms mean that meropenem takes longer to encounter resistance compared to other antibiotics.[13]

Comparison of the meropenem and meropenem amikacin combination groups obtained a normality test significance. $p<0.05$, so that the data is not normally distributed. The result is $p>0.05$ for the homogeneity test, which means the data are homogeneous. Then, the Kruskal-Wallis non-parametric comparison test with a significance value was continued. 0.366 ($p>0.05$) means there is no significant difference between single meropenem administration compared to the combination in this study. This can be seen from the range of resistance time between the two groups, which is not much different. So, that in vitro *Klebsiella pneumoniae ESBL* antibiotic combination is not better than single meropenem.

The limitations of this study are that most of the specimens taken from ICU patients had already been given antibiotic therapy. The characteristics of the specimens used in this study were not homogeneous, including the history of antibiotic administration for each sample, which was not the same, and the duration of antibiotic administration was not explained in detail in the patient's medical record before specimen collection, which would affect the duration of resistance. In addition, samples were not subjected to genotypic examination at the beginning and end of the study, so the cause of drug resistance is unknown. This study carried out treatments up to 14 times, but if the inhibition zone had reached resistance, then the treatment was stopped, so the antimicrobial effect after resistance occurred was unknown. In addition, this study did not administer various doses of antibiotic combinations.

CONCLUSIONS

Klebsiella pneumonia ESBL resistance progressed in vitro to meropenem and amikacin therapy in clinical specimens from the Saiful Anwar Hospital Malang Intensive Care Unit. In vitro administration of amikacin antibiotics against *Klebsiella pneumonia ESBL* bacteria showed resistance on the 5th day of exposure, meropenem showed resistance on the 12th day of exposure, and the combination antibiotic amikacin meropenem showed resistance on the 14th day of exposure. There was no significant difference in the duration of monotherapy resistance and the combination of meropenem and amikacin antibiotics against *Klebsiella pneumonia ESBL* bacteria.

DISCLOSURES

FUNDING

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ETHICAL CLEARANCE

This study has obtained ethical clearance from the Ethics Committee of the Faculty of Medicine, Brawijaya University, with reference letter number 100/EC/KEPK/05/2023.

CONFLICT OF INTEREST

The authors affirm that there are no conflicts of interest in this study.

AUTHOR CONTRIBUTION

All authors contributed equally to this manuscript's research and publication, and we agree to submit the final version to this journal.

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