DOI:10.34084/bshr.1590089 e-ISSN 2587-1641

Journal of Biotechnology and Strategic Health Research

Araştırma Makalesi /Research Article

http://dergipark.org.tr/tr/pub/bshr

Evaluation of Meropenem and Gentamicin Synergy on *Klebsiella pneumonaie*

Meropenem ve Gentamisin Sinerjisinin *Klebsiella pneumoniae* Üzerinde Değerlendirilmesi

Ali Ünal[1], Yeliz Tanrıverdi Çaycı[2], İlknur Bıyık[3]

[1] Ondokuz Mayıs Üniversitesi, Tıp Fakültesi, Tıbbi Mikrobiyoloji Anabilim Dalı, Samsun, Türkiye

[2] Ondokuz Mayıs Üniversitesi, Tıp Fakültesi, Tıbbi Mikrobiyoloji Anabilim Dalı, Samsun, Türkiye

[3] Ondokuz Mayıs Üniversitesi, Tıp Fakültesi, Tıbbi Mikrobiyoloji Anabilim Dalı, Samsun, Türkiye

ORCID ID: Ali Ünal: https://orcid.org/0009-0006-1014-4448, Yeliz Tanrıverdi Çaycı: https://orcid.org/0000-0002-9251-1953, İlknur Bıyık: https://orcid.org/0000-0002-3247-883X

***Sorumlu Yazar / Corresponding Author:** Ali Ünal, **e-posta / e-mail:** ali_unaal@hotmail.com

Geliş Tarihi / Received : 23-11-2024 **Kabul Tarihi / Accepted:** 27-11-2024 **Yayın Tarihi / Online Published:** 31-12-2024

Ünal A., Tanrıverdi Çaycı Y., Bıyık İ., Evaluation of Meropenem and Gentamicin Synergy on Klebsiella pneumonaie. J Biotechnol and Strategic Health Res. 2024;8(3):236-241

Abstract

Amaç Antimikrobiyal direnç, hem tıbbi hem de ekonomik sonuçlara neden olan ciddi bir küresel sorundur. *Klebsiella pneumoniae (K. pneumoniae*), yaygın direnç mekanizmaları nedeniyle bu yüklerde önemli rol oynayan gram-negatif bir bakteridir. Bu çalışma, disk difüzyon yöntemini kullanarak meropenem ve gentamisin arasındaki sinerji ve antagonizmi araştırmakta ve bu bulguları dama tahtası yönteminden elde edilen sonuçlarla karşılaştırmaktadır. Sonuçlar tutarlıysa, disk difüzyon yönteminin güvenilirliğini doğrulayarak, diğer test yöntemlerine uygun maliyetli bir alternatif

Gereç ve Yöntem Dama tahtası yöntemi, iki antimikrobiyal ajanın etkileşimini (sinerjik, kayıtsız veya antagonistik) değerlendirmek için, ajanların değişen konsantrasyonlarını içeren kuyucuklardan oluşan bir ızgara hazırlayarak kullanılır. Kombinasyonun etkinliğini değerlendirmek için fraksiyonel inhibitör konsantrasyon indeksi (FICI) hesaplanır.
Disk difüzyon yönteminde, bir ajanın antimikrobiyal aktivitesi, ajana batırılmış disklerin bakterilerle aşılanmış bir agar plakasına yerleştirilmesiyle belirlenir. İnkübasyondan sonra, duyarlılığı değerlendirmek için inhibisyon bölgesinin çapı ölçülür.

Bulgular Her iki yöntemle de 30 Klebsiella pneumaniae izolatının hiçbirinde sinerji veya antagonizm tespit edilmemiştir.

Sonuç Disk difüzyon yöntemi, özellikle imkanların kısıtlı olduğu durumlarda, diğer in vitro sinerji testlerine uygun maliyetli ve güvenilir bir alternatiftir.

Anahtar Kelimeler gentamisin, in vitro sinerji, *Klebsiella Pneumoniae*, meropenem

Özet

Aim *Antimicrobial resistance is a severe global problem, causing both medical and economic results. Klebsiella pneumoniae (K. pneumoniae) is a gram-negative bacterium that plays a major role in these burdens due to its widespread resistance mechanisms. This study searchs for the synergy and antagonism between meropenem and gentamicin using the disk diffusion method and compares these findings with results from the checkerboard method. If the results are consistent, validating the reliability of the disk diffusion method, it could be a cost-effective alternative to other testing*

Material and Method *The checkerboard method is used to assess the interaction of two antimicrobial agents (synergistic, indifferent, or antagonistic) by preparing a grid of wells containing varying concentrations of the agents. The fractional inhibitory concentration index (FICI) is calculated to evaluate the combination's efficacy.*
In the disc diffusion method, the antimicrobial activity of an agent is determined by placing discs soaked in the agent on an agar plate inoculated with bacteria. After incubation, the diameter of the inhibition zone is measured to assess susceptibility.

Results *No synergy or antagonism was detected in any of the 30 Klebsiella pneumaniae isolates by either method.*

Conclusion *The disc diffusion method is a cost-effective and reliable alternative to other in vitro synergy tests, especially when facilities are limited.*

Keywords *gentamicin, in vitro synergy, Klebsiella Pneumoniae, meropenem*

INTRODUCTION

Klebsiella pneumoniae is a gram-negative bacterium that may be localized in various sites. In animals, it is found on mucosal surfaces and in environmental locations such as water and soil. In humans, it can also be found in the gastrointestinal tract and nasopharynx.[1] However, pathogenic strains of *K. pneumoniae* create significant challenges in treatment, due to the variety of their resistance mechanisms. These are extended-spectrum beta-lactamases (ESBLs) and carbapenemases, which are responsible for resistance to many beta-lactam antibiotics, and plasmid-mediated AmpC enzymes promote multidrug resistance, complicating treatment options.[2,3] Some clinical *K. pneumoniae* isolates are resistant to all available antibiotics.[4]

The increase in these multidrug-resistant isolates necessitates the development of new classes of antibiotics or alternative treatment strategies.[5] Especially in populations such as immunocompromised patients or those with underlying conditions, infection can lead to serious diseases resulting in high morbidity and mortality rates.[6,7] Resistance of *K. pneumoniae* to broad-spectrum antibiotics significantly limits treatment choices and leads to increased healthcare costs, longer hospital stays, and the need for more expensive treatments.[8] As a possible treatment for these infections, this study aims to detect if there is synergy between meropenem, a widely used broad-spectrum carbapenem, and gentamicin, an aminoglycoside. This study aims to determine whether using of these antibiotics in combination is more effective against resistant *K. pneumoniae* strains. The reason for selecting these two antibiotics is based on previous studies showing that carbapenem-resistant *Klebsiella pneumoniae* (CRKP) strains resistant to meropenem may exhibit susceptibility to gentamicin.[9] Additionally, some studies have demonstrated a potential synergy between meropenem and gentamicin in certain cases.[10] Furthermore, gentamicin-based combinations showed a high level of synergy determined in the study conducted by Oliva et al.[11] The primary aim of this study, in addition to identifying the potential synergy between these two selected antibiotics, is to evaluate the reliability of the disk diffusion method. In vitro synergy tests are highly costly procedures. Therefore, assessing the reliability of the disk diffusion method holds significant importance for determining antibiotic combinations in healthcare centers lacking access to such facilities. This study will further compare the outcomes of both methods to establish whether the disk diffusion method, while easy and accessible, gives results comparable to the more complex checkerboard method. If validated, the disk diffusion method may serve as a reliable alternative in resource-limited settings and may make treatment selection more cost-effective in the case of antimicrobial resistance.

METHODOLOGY

Thirty carbapenem-resistant Klebsiella pneumoniae (CRKP) isolates obtained from samples sent to the Ondokuz Mayıs University Microbiology Laboratory were included in the study. The identification of the isolates was performed using the Vitek MS (Biomeriux, France) system, and antibiotic susceptibility was determined with the Vitek2 Compact (Biomeriux, France) device. Two different methods were used to evaluate synergy between meropenem and gentamicin: the disk diffusion method and the checkerboard method. The disk diffusion method is simpler and more cost-effective, while the checkerboard technique, which is complex and more costly. Initially, the inhibition zone diameters of all isolates were determined by the disk diffusion method. Additionally, the minimum inhibitory concentration (MIC) values of all isolates were determined using the microdilution method in a 96-well plate.

Synergy with Disk Diffusion

A bacterial suspension was prepared from the isolate to be tested at a bacterial density of 0.5 McFarland, and then it was inoculated onto a Mueller-Hinton agar plate. Meropenem and gentamicin disks were placed 20 mm apart, and the plate was incubated at 37°C for 20 hours. Following

incubation, the presence or absence of bridging between the disks and a comparison with the initial zone diameter were evaluated. An increase of ≥2 mm in the inhibition zone diameter or the presence of bridging was interpreted as synergy.[12]

Checkerboard Method

Stock solutions of each antibiotic were prepared for the synergy test, and two-fold serial dilutions were performed in separate tubes. In each well designated for combination testing, twice the desired final concentration was prepared in the tube, as both antibiotics were present in equal amounts in the same well, diluting each other by one-fold.

- Each well designated for the combination was given 50 µL from each of the two antibiotic solutions, resulting in a final volume of 100 µL per well.
- In each microplate panel, the first horizontal row (A2-A12) and the first vertical column (B1-H1) wells were reserved for determining the MICs of individual antibiotics (antibiotics a and b). The other wells contained the combinations of antibiotics a + b.
- While preparing the antibiotic dilutions to be transferred to the wells (at a higher concentration), the volume of solution was calculated as the number of wells × 50 µL per well. For instance, if the desired concentration of antibiotic a in the well was 0.5 µg/mL, then the concentration prepared in the tube was 1 µg/mL, and the volume was calculated as the number of wells that required 0.5 µg/mL of antibiotic a × 50 µL (e.g., 8 wells × 50 µL = 400 µL in the tube).
- A bacterial suspension with a turbidity of 0.5 McFarland (1.5 × 10^8 CFU/mL) was prepared and diluted at a ratio of 1:30 (5 × 10^6 CFU/mL); 10 µL (5 × 10^4 CFU) was then inoculated into each well, except for the sterility control well. Thus, each well with 100 µL of antibiotic combination solution contained approximately 5 × 10^4 CFU/100 µL, or 5 × 10^5 CFU/mL of bacteria.
- If Mueller-Hinton Broth (MHB) was used in the bacterial suspension, the antibiotic dilutions were also prepared in MHB.
- In each experiment, one well (A1) was left without antibiotics and used as a growth control. During reading, this well was expected to exhibit heavy turbidity.
- One well (H12) contained only medium and served as the medium sterility control.
- After the panel was completed, the microplate lid was closed and incubated for 16-20 hours at 35-37°C. At the end of incubation, wells without growth were identified, and the Fractional Inhibitory Concentration (FIC) index was calculated to determine synergy, which was then compared with the results of the disk diffusion synergy test.

If the FIC index was ≤0.5, it was evaluated as synergy; between 1 and 4 as indifference; and >4 as antagonism.[13]

The results obtained with the disk diffusion synergy method were compared with those obtained by the checkerboard method.

DISCUSSION

In this study, no synergism between meropenem and gentamicin was found same as previous studies.[14] However, there are no further studies investigating the interaction between meropenem and gentamicin. This emphasises the continuing challenges in the treatment of infections caused by carbapenem-resistant *Klebsiella pneumoniae* (CRKP). Furthermore, this observation is in line with previous studies that underline the disc diffusion method as a reliable test.[15,16] Similarly, antimicrobial susceptibility results determined using the broth microdilution method and disc diffusion test results were found to be compatible.[17] CRKP is known for its ability to develop multidrug resistance due to mechanisms such as carbapenemase production, extended-spectrum beta-lactamases (ESBLs), efflux pumps and alterations in membrane permeability.[18,19,20,21] These factors make it difficult to develop effective combination therapies and it has become peremptory to develop

treatment options as an alternative to antibiotic therapy for combating antibiotic-resistant pathogens.[22]

The consistency of the results obtained from both methods in this study emphasizes the reliability of the disc diffusion technique, which is an in vitro synergy test. This research contributes to the validation of the disk diffusion method, supporting its potential as a preliminary tool for detecting antibiotic synergy, especially in resource-limited settings. The disk diffusion method offers a more accessible and cost-effective alternative to the time- consuming and resource-intensive checkerboard method. The use of disk diffusion for initial screening may be useful in healthcare centre that do not have advanced microbiological testing and may allow timely definition of resistance profiles and identification of the appropriate antibiotic.[23] This procedure can help rapidly assess antibiotic effectiveness, especially in developing countries where resistance testing resources are limited.

The consistency of the results obtained from both methods in this study highlights the reliability of the disk diffusion technique, an in vitro synergy test. The consistency of the results obtained from both methods in this report justifies the disk diffusion technique as a reliable in vitro synergy test. This study contributes to the validation of the disk diffusion method, supporting its potential as a preliminary tool for the detection of antibiotic synergy, especially in resource-limited settings. The disk diffusion method offers a more accessible and cheaper alternative to the checkerboard method.[24] The use of disk diffusion for initial screening may be useful in healthcare facilities that do not have advanced microbiological testing infrastructure and may allow for timely determination of resistance profiles and identification of the correct antibiotic. This technic may offer the possibility of a rapid way of evaluating antibiotic efficiency, especially in developing and low-income countries where resistance-testing resources are limited.[25,26]

In conclusion, no synergy or antagonism detected between meropenem and gentamicin against CRKP, as determined with both disk diffusion and checkerboard methods, underscores the complexity of treating infections caused by multidrug-resistant organisms. The validation of this study the disk diffusion method as a reliable tool in resource-limited settings represents a significant advancement in the fight against antimicrobial resistance, by making access easier for assessing resistance patterns.

However, unlike our findings, some studies have mentioned a synergistic interaction between meropenem and gentamicin.[26,27] These contrasting results highlight the need for investigation in search of more appropriate combination alternatives, new antibiotic drugs, and novel therapeutic approaches. Considering ongoing increases in worldwide antimicrobial resistance, clinical practice needs to evolve and include advances in diagnosis, as well as evidence-based programs, to enhance treatment outcomes and limit the spread of resistant pathogens. With collaboration in research and adapting healthcare practices, the medical community can work toward sustainable solutions in the management of infections caused by such challenging pathogens as CRKP.

RESULTS

The disc diffusion method showed that there was no synergy between meropenem and gentamicin in any of the 30 *K. pneumoniae* isolates tested. The fact that the zones of inhibition remained below the 2 mm increase required to show synergy proves that the antibiotics do not enhance the effects of each other under these conditions.

The results of the checkerboard method were consistent with the findings of the disc diffusion method for all 30 isolates. FICI values in all combinations were above 0.5, indicating no synergy. In addition, no antagonistic interaction (FICI > 4) was observed. The disc diffusion and checkerboard methods gave the same results, indicating that there was no synergy between meropenem and gentamicin for the *K. pneumoniae* isolates tested.

CONCLUSION

This study confirms that there is no synergy exists between meropenem and gentamicin against *K. pneumoniae* isolates based on both the disk diffusion and checkerboard methods. The consistency in results between these methods validates the disk diffusion method as a preliminary screening tool, especially in settings with limited resources, cost-effective approach for the initial testing of antibiotic combinations. Further studies are recommended using larger sample sizes with different antibiotic combinations to find effective treatments against resistant *K. pneumoniae* strains.

Informed Consent

The patients were sampled through convenient sampling technique and enrolled after obtaining their written informed consent.

Peer-review

Externally and internally peer-reviewed.

Authorship Contributions

Concept: A.Ü., Y.T.Ç, İ.B., Design: A.Ü., Y.T.Ç., İ.B., Data Collection or Processing: Y.T.Ç., İ.B., Analysis and Interpretation: A.Ü., Y.T.Ç., İ.B., Literature Search: A.Ü., Writing: A.Ü., Conflict of Interest: No conflict of interest was declared by the authors.

Conflict of Interest

No conflict of interest was declared by the authors.

Funding

The authors declared that this study received financial support from TUBITAK-2209 (Number: 1919B012216444).

References

1. Guoying W, Guo Z, Xiaoyu C, Longxiang X, Hongju W. the characteristic of virulence, biofilm and antibiotic resistance of klebsiella pneumoniae. int j environ res public health. 2020; 17(17): 6278. DOI: 10.3390/ijerph17176278
2. Baykal A, Cöplü N, Simsek H, Esen B, Gür D. Kan İzolatı E. coli ve K.pneumoniae suşlarında genişlemiş spektrumlu beta-laktamaz, kpc-tip karbapenemaz ve plazmid aracılı ampc beta-laktamaz varlığının araştırılması. mikrobiyol bul. 2012; 46(2): 159-69. PMid: 22639305
3. Karampatakis, T.; Tsergouli, K.; Behzadi, P. Carbapenem-Resistant Klebsiella pneumoniae: virulence factors, molecular epidemiology and latest updates in treatment options. antibiotics 2023, 12, 234. DOI: 10.3390/antibiotics12020234
4. Elemam, J. Rahimian, W. Mandell. infection with panresistant klebsiella pneumoniae: a report of 2 cases and a brief review of the literature. Clinical Infectious Diseases, Volume 49, Issue 2, 15 July 2009, Pages 271–274. DOI: 10.1086/600042
5. Dennis J. Doorduijn, Suzan H.M. Rooijakkers, Willem van Schaik, Bart W. Bardoel. complement resistance mechanisms of klebsiella pneumoniae. immunobiology. volume 221, issue 10, october 2016, pages 1102-1109. DOI: 10.1016/j.imbio.2016.06.014
6. Giske CG, Monnet DL, Cars O, Carmeli Y. clinical and economic impact of common multidrug-resistant gram-negative bacilli. Antimicrob Agents Chemother. 2008;52(3):813–21. DOI: 10.1128/AAC.01169-07
7. Martin, R.M.; Bachman, M.A. colonization, infection, and the accessory genome of klebsiella pneumoniae. front. cell. infect. microbiol. 2018, 8, 4. DOI: 10.3389/fcimb.2018.00004
8. Havan M, Kendirli T, Parlar ÖT, et al. clinical management of a pandrug-resistant oxa-48 klebsiella pneumoniae infection in the pediatric intensive care unit. microb drug resist. 2023 Jun;29(6):256-262. DOI: 10.1089/mdr.2022.0247
9. Cojutti P, Sartor A, Bassetti M, Scarparo C, Pea F. is meropenem mic increase against kpc-producing klebsiella pneumoniae correlated with increased resistance rates against other antimicrobials with gram-negative activity? j glob antimicrob resist. 2018 sep;14:238-241. doi: 10.1016/j.jgar.2018.05.005. Epub 2018 Jul 9. DOI: 10.1016/j.jgar.2018.05.005
10. Firmo EF, Oliveira Júnior JB, Scavuzzi AML, et al. in vitro activity of polymyxin b in combination with meropenem, amikacin and gentamicin against Klebsiella pneumoniae clinical isolates co-harbouring aminoglycoside-modifying enzymes, blaNDM-1 and blaKPC-2. J Glob Antimicrob Resist. 2020 Sep;22:511-514. doi: 10.1016/j.jgar.2020.04.014.
11. Oliva A, Scorzolini L, Cipolla A, et al. in vitro evaluation of different antimicrobial combinations against carbapenemase-producing Klebsiella pneumoniae: the activity of the double-carbapenem regimen is related to meropenem MIC value. J Antimicrob Chemother. 2017 Jul 1;72(7):1981-1984. DOI: 10.1093/jac/dkx084
12. Chen Z, Xiang J. preliminary study on resistance mechanism and virulence features in carbapenems-resistant Klebsiella pneumoniae from burn patients. Zhonghua Shao Shang Za Zhi. 2018 Nov 20;34(11):796-801. Chinese. doi: 10.3760/cma.j.issn.1009-2587.2018.11.015.
13. Yu L, Zhang J, Fu Y, et al. synergetic effects of combined treatment of colistin with meropenem or amikacin on carbapenem-resistant klebsiella pneumoniae in vitro. front cell infect microbiol. 2019 Dec 10;9:422. doi: 10.3389/fcimb.2019.00422.
14. Toledo PV, Tuon FF, Arend L, Aranha Junior AA. efficacy of tigecycline, polymyxin, gentamicin, meropenem and associations in experimental Klebsiella pneumoniae carbapenemase-producing Klebsiella pneumoniae non- lethal sepsis. Braz J Infect Dis. 2014 Sep-Oct;18(5):574-5. DOI: 10.1016/j.bjid.2014.05.003
15. Gaudereto JJ, Neto LVP, Leite GC, Espinoza EPS, Martins RCR, villas boa prado g, rossi f, guimarães t, levin as, costa sf. comparison of methods for the detection of in vitro synergy in multidrug-resistant gram-negative bacteria. bmc microbiol. 2020 Apr 16;20(1):97. doi: 10.1186/s12866-020-01756-0.
16. Matuschek E, Copsey-Mawer S, Petersson S, Åhman J, Morris TE, Kahlmeter G. the european committee on antimicrobial susceptibility testing disc diffusion susceptibility testing method for frequently isolated anaerobic bacteria. Clin Microbiol Infect. 2023 Jun;29(6):795.e1-795.e7. DOI: 10.1016/j.cmi.2023.01.027
17. Erinmez M, Zer Y. In vitro effects of deferoxamine on antibiotic susceptibility in Gram-negative bacteria. Adv Clin Exp Med. 2024 May;33(5):491-497. doi: 10.17219/acem/169794.
18. Nordmann, P.; Naas, T.; Poirel, L. global spread of carbapenemase-producing Enterobacteriaceae. Emerg. Infect. Dis. 2011, 17, 1791–1798. DOI: 10.3201/eid1710.110655
19. Foudraine, D.E.; Strepis, N.; Klaassen, C.H.W. et al. rapid and accurate detection of aminoglycoside-modifying enzymes and 16s rrna methyltransferases by targeted liquid chromatography-tandem mass spectrometry. J. Clin Microbiol. 2021. DOI: 10.1128/JCM.00464-21
20. Doménech-Sánchez, A.; Martínez-Martínez, L.; Hernández-Allés, S., et al. Role of Klebsiella pneumoniae OmpK35 porin in antimicrobial resistance. Antimicrob. Agents Chemother. 2003, 47, 3332–3335. DOI: 10.1128/AAC.47.10.3332-3335.2003
21. Srinivasan, V.B.; Singh, B.B.; Priyadarshi, N.; Chauhan, N.K.; Rajamohan, G. Role of novel multidrug efflux pump involved in drug resistance in Klebsiella pneumoniae. PLoS ONE 2014, 9. DOI: 10.1371/journal.pone.0096288
22. Bhardwaj S, Mehra P, Dhanjal DS, et al. antibiotics and antibiotic resistance flipsides of the same coin. curr pharm des. 2022;28(28):2312-2329. DOI: 10.2174/1381612828666220608120238
23. Jeannot K, Gaillot S, Triponney P, Portets S, Pourchet V, Fournier D, Potron A. performance of the disc diffusion method, mts gradient tests and two commercially available microdilution tests for the determination of cefiderocol susceptibility in acinetobacter spp. microorganisms. 2023 Jul 31;11(8):1971. DOI: 10.3390/microorganisms11081971
24. Mansour-Ghanaei F, Poostizadeh G, Joukar F, Siavoshi F. efficacy of disc diffusion and agar dilution methods in evaluating helicobacter pylori susceptibility to antibiotics. middle east j dig dis. 2022 Apr;14(2):207-213. DOI: 10.34172/mejdd.2022.274
25. Khaki P, Sharma A, Bhalla P. comparison of two-disc diffusion methods with minimum inhibitory concentration for antimicrobial susceptibility testing of neisseria gonorrhoea isolates. ann med health sci res. 2014 May;4(3):453-6. DOI: 10.4103/2141-9248.133477
26. Rogers TM, Kline EG, Griffith MP, et al. mutations in ompk36 differentially impact in vitro synergy of meropenem/vaborbactam and ceftazidime/avibactam in combination with other antibiotics against kpc-producing Klebsiella pneumoniae. JAC-antimicrobial resistance. 2023 Oct 26;5(5): dlad113. DOI: 10.1093/jacamr/dlad113
27. Dobreva E, Ivanov I, Donchev D, et al. in vitro investigation of antibiotic combinations against multi- and extensively drug-resistant Klebsiella pneumoniae. Open Access Maced J Med Sci [Internet]. 2022 Apr. 5;10(B):1308-14.