Open Access

Original Article

In Vitro Efficacy of Imipenem/Relebactam Against Imipenem Resistant Gram-Negative Strains Isolated from a Tertiary Care Hospital

Saima Ishtiaq, Sonia Tahir*, Muhammad Waqas Abbas, Haider Ali, Saima Syed, Fatima Kareem**

Department of Microbiology, Foundation University School of Health, Islamabad Pakistan, *Department of Microbiology, Lahore Medical & Dental College, Lahore Pakistan, **Department of Microbiology, Fauji Foundation Hospital, Rawalpindi Pakistan

ABSTRACT

Objective: To determine in vitro efficacy of Imipenem/Relebactam against multi-drug resistant strains.
Study Design: Cross-sectional study.
Place and Duration of Study: Department of Microbiology, Fauji Foundation Hospital (FFH) Rawalpindi, Pakistan from Jul to Dec 2022.
Methodology: All specimens from indoor and outdoor patients with infection due to Gram-negative organisms showing resistance to Imipenem disc (10µg) were included. Cultures were inoculated on Blood and MacConkey's agar. Imipenem-resistant Gram-negative species identification was made by Gram stain, Oxidase test and commercially available identification strips Analytical profile index 10S (API-10S) (from bioMerieux, Inc.).Imipenem-resistant species were identified, showing a zone diameter of less than 23mm against10µg Imipenem disc for Enterobacterale and Non-Enterobacterales, and for Pseudomonas species, zone size is ≤19mm. These resistant organisms were tested for sensitivity against Imipenem/Relebactam by E-strip (MTSTM –MIC Test Strip) (Liofilchem, Inc., Waltham, MA).
Results: Out of 160 Carbapenem-resistant Enterobacterale and Non-Enterobacterales specimens, only 14 isolates (including 02 Pseudomonas spp.) showed susceptibility to Imipenem/Relebactam combination of drug. US-FDA Imipenem/Relebactam susceptibility interpretive criteria (STIC) was used.
Conclusion: In our setup, only 8.75% isolates showed sensitivity to this newer drug combination Imipenem/Relebactum. This highlights the importance of evaluating newer drugs in our population before using them empirically.

Keywords: Carbapenems, Imipenem, Anti-bacterial agents, Drug resistance, Bacterial drug resistance, Multiple drug resistance.

How to Cite This Article: *Ishtiaq S, Tahir S, Abbas MW, Ali H, Syed S, Kareem F. In Vitro Efficacy of Imipenem/Relebactam Against Imipenem Resistant Gram-Negative Strains Isolated from a Tertiary Care Hospital. Pak Armed Forces Med J 2024; 74(1): 94-98.*
DOI: https://doi.org/10.51253/pafmj.v74i1.9901

INTRODUCTION

Highly resistant organisms are becoming more and more prevalent, and we are left with fewer treatment options. There are the number of factors involved in causing this situation of drug resistance.[1,2] Over-use and misuse of antibiotics, not practising regular de-escalation of drugs for chronically ill patients taking these antibiotics, and use of multiple antimicrobial combinations as empirical treatments and prescribing them without consulting their sensitivities.[3] To overcome the problem of fewer treatment options for chronically ill patients with drug-resistant organisms, medical scientists are constantly working on providing some effective treatment options and introducing newer drugs every now and then.[4]

Imipenem, a β-lactam drug, is a carbapenem moiety of antibiotics. It is spectrum of activity is broader compared to other β-lactam drugs like Penicillins and Cephalosporins.[5] It was the drug of choice for severalseveral medical conditions complicated by gram-positive or gram-negative organisms. Imipenem was taken as the drug of last resort for patients with complicated urinary tract infections, complicated intra-abdominal infections, lung infections, and various skin and soft tissue infections. It was used in combination with other antimicrobial classes such as aminoglycosides or glycopeptides.[6,7]

Several drug combinations have been tried and tested such as Piperacillin/Tazobactam, Ceftazidime/Avibactam and Meropenem Vabor-bactam. They have shown promising results for the treatment of complicated cases of urinary tract infections, intra-abdominal infections and bacteremia caused by gram-negative organisms.[8] Imipenem-relebactam is an addition to such assets of antimicrobials, especially for the treatment of multi-

Correspondence: Dr Saima Ishtiaq, Department of Microbiology, Foundation University School of Health, Islamabad Pakistan
Received: 06 Feb 2023, revision received: 24 May 2023; accepted: 25 May 2023

drug resistant organisms.[9,10] Imipenem is a β-lactam drug and relebactam is a β-lactamase inhibitor. Together, they work synergistically to improve the bactericidal effect of Imipenem by sparing it from β-lactamases. The study aims to assess the in vitro efficacy of Imipenem/Relebactam against multi-drug resistant strains, providing valuable insights into the susceptibility of Gram-negative organisms to this drug combination. The study findings contribute to informed decision-making for empirical treatment in our population, emphasizing the need for local assessments of newer drugs.

METHODOLOGY

The cross-sectional study was conducted at the Department of Microbiology, Fauji Foundation Hospital, (FFH) Rawalpindi Pakistan, from July to December 2022, after approval from the Ethical Review Board (No. 510/RC/FFH/RWP). Sample size was calculated using the WHO sample size calculator, taking a confidence the reported proportion of antibiotic sensitivity of 33.5%.[11]

Inclusion Criteria: All indoor and outdoor patients aged 12-60 years, of either gender with infections due to gram-negative organisms, showing resistance for Imipenem as a disc zone size of ≥23mm and for Pseudomonas zone size of ≥19mm were included.

Exclusion Criteria: Patients with infections due to gram-negative organisms, showing antibiotic sensitivity for Imipenem as a disc zone size of <23mm and for Pseudomonas zone size of <19mm were excluded.

A total of 160 Carbapenem-resistant Enterobacterale and Non-Enterobacterales isolates were checked for the the Imipenem/Relebactam drug combination sensitivity. Cultures were collected in the Microbiology department FFH and were inoculated on Blood agar and McConkey agar. These plates were incubated for 24-48 hours at 37°C. Gram-negative species identification was done by Gram stain for morphology and oxidase test and was further confirmed by commercially available identification strips, Analytical profile index 10S(API-10S)(bioMerieux, Inc. USA).

Further, these species were identified for Imipenem resistance by inoculating these Gram-negative species on the Mueller Hinton agar plate. According to a recommendation given by CLSI 2022, the antibiotic panel was placed. The plates were incubated for 18-24 hours at 37°C and were read for antibiotic sensitivities. The organisms showing a zone size of less than 23mm (and for Pseudomonas, zone size was ≤19mm) against Imipenem disc(10µg) were selected for the study.

E-strips tested these resistant organisms for Minimal inhibitory concentration (MIC) of Imipenem/Relebactam. MIC of the Imipenem/Relebactam isolates were obtained using E strips (MTSTM –MIC Test Strip) (Liofilchem, Inc., Waltham, MA). These E-strips were stored at -20 °C as guided by the manufacturer. Fresh growth, within 24 hours of carbapenem-resistant Enterobacterale and Non-Enterobacterales, was used to inoculate on muller hinton agar. ATCC Quality control strains were also inoculated with them. Imipenem/Relebactam concentration E-strips of 0.002/4-32/4 µg/ml were used. The inoculated plates were read after 18-24 hours of incubation. The elliptical zone of MIC was read according to US FDA susceptibility interpretive criteria (STIC).[12]

Statistical Package for Social Sciences (SPSS) version 20.0 was used for the data analysis. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages.

RESULTS

A total of 160 Carbapenem-resistant strains of Enterobacterale and Non-Enterobacterales were included in the study. These were isolated from pus, urine, respiratory tract, blood, CSF, and fluid samples. Table-I shows the different types of samples included in the study. Pus samples were most frequently used, comprising 40% of total samples, followed by urine and respiratory tract samples, 26.5% and 25% of total samples, respectively. Cerebrospinal fluid (CSF) and ascitic fluid were the least common, accounting for only 2.5% of the sample.

Table-I: Types of Samples Included in Study (n=160)

Types of samples	n (%)
Pus	64(40%)
Urine	42(26.25%)
Respiratory Tract	40(25%)
Blood	10(6.25%)
CSF and Ascitic Fluid	04(2.5%)
Total	160(100%)

Of 160 carbapenem-resistant strains, the most common isolated organism was *Pseudomonas* species, making 38.75% of all isolates. This was followed by *Escherichia coli and Acinetobacter baumannii,*

constituting 25% and 17.5% of all isolates, respectively. *Enterobacter* species were least commonly isolated, having only 1.25% representation.

Only 14 isolates (8.75%) showed susceptibility to Imipenem/Relebactam. Among *Pseudomonas* species, 04 out of 62(6.45%) were sensitive to Imipenem/Relebactam, whereas 02 out of 40(5%) isolates of *Escherichia coli* were susceptible to Imipenem/Relebactam. Table-II shows the distribution of various isolated species and their sensitivity to Imipenem/Relebactam. *Providencia* isolates showed the lowest MICs, whereas isolates of *Acinetobacter and Pseudomonas* species displayed the highest MICs. Table-III depicts the range of MICs of Imipenem/relebactam 0.002/4-32/4 µg/ml E-strip for susceptible organisms.

Table-II: Organisms Isolated and Susceptibility of Carbapenem Resistant Isolates to Imipenem/Relebactam E-strips (n=160)

Organisms	Carbapenem resistant Enterobacterales and Non-Enterobacterales n (%)	Susceptible to Imipenem/ Relebactam E-strip n (%)
Pseudomonas species	62(38.75%)	04(2.5%)
Escherichia coli	40(25%)	02(1.25%)
Klebsiella pneumoniae	10(6.25%)	00
Klebsiella oxytoca	12(7.5%)	00
Acinetobacter baumannii	28(17.5%)	02(1.25%)
Enterobacter species	02(1.25%)	02(1.25%)
Providencia species	02(1.25%)	02(1.25%)
Serratia marcescens	04(2.5%)	02(1.25%)
Total	160(100%)	14(8.75%)

DISCUSSION

The emergence of MDR gram-negative bacteria has become a significant challenge in treating infections. Imipenem/relebactam is a new weapon in the armamentarium of antimicrobials.[12,13] FDA approved it in 2019 for adults with complicated intra-abdominal and urinary tract infections.[14]

Our study conducted in Rawalpindi, at a tertiary level hospital, showed that the newer drug combination Imipenem/Relebactam showed no promising results. Out of 160 Carbapenem-resistant isolates, only 8.75% showed susceptibility to Imipenem/Relebactam. This finding contrasts the studies conducted in various regions of Europe and America. In a study by Lob *et al*. conducted in Europe from 2015-2017, the addition of relebactam makes 42-75% of Imipenem-resistant gram-negative isolates susceptible.[12] Similarly, a study conducted in Japan by Kurihara *et al*. showed higher (around 70%) sensitivity to a combination of Imipenem- Relebactam in Imipenem-resistant gram-negative isolates.[10] A notable finding of the Japanese study was the sensitivity of 100% isolates of *Acinetobacterbaumannii to* Imipenem-relebactam, whereas only 2 out of 28(7.1%) *Acinetobacter baumannii* strains showed susceptibility in our study.

This marked difference in sensitivity to Imipenem- Relebactam may be related to different genetic makeup of bacteria where the prevalence of drug-resistant genes may vary with geographical variations.[15-18]

All newer drugs must be evaluated in vitro and in

Table-III: Range of MICs of Imipenem/Relebactam 0.002/4-32/4 µg/ml E-strip for Susceptible Organisms

Organisms	8 µg/ml	6 µg/ml	4 µg/ml	3 µg/ml	2 µg/ml	1.5 µg/ml	1 µg/ml	0.75 µg/ml	0.50 µg/ml	0.38 µg/ml	0.25 µg/ml	0.19 µg/ml	0.125 µg/ml
Pseudomonas							1						
Pseudomonas								0.75					
Pseudomonas							1						
Pseudomonas								0.75					
E.coli							1						
E.coli							1						
Acinetobacter					2								
Acinetobacter					2								
Enterobacter								0.75					
Enterobacter									0.50				
Providencia													0.125
Providencia												0.19	
Serratia											0.25		
Serratia										0.38			

vivo to be used in our setup, according to the genetic setup of bacterial isolates.

Nevertheless, our study has highlighted that the antimicrobial sensitivity pattern is unique in our part of the world. More local studies are needed to establish and update Pakistan's antimicrobial stewardship guidelines. Our study is the first to evaluate in vitro efficacy of Imipenem-Relebactam in Pakistan.

LIMITATION OF STUDY

One of the limitations of our study was that genetic analysis was not performed in resistant strains. Our study was conducted in the region of Punjab, so a review of the general population could not be given.

CONCLUSION

In contrast to foreign data, the combination Imipenem/Relebactam did not show in vitro efficacy against MDR gram-negative isolates. In our setup, newer drug combinations should always be evaluated regarding their efficiency and sensitivity patterns. First, we should always know their effectiveness as MDR bacterial strains with different genetic configurations exist in different geographical locations. Their sensitivity pattern shows diverse variability according to different regions.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

SI & ST: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

MWA & HA: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

SS & FK: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Vivas R, Barbosa AAT, Dolabela SS, Jain S. Multidrug-Resistant Bacteria and Alternative Methods to Control Them: An Overview. Microb Drug Resist 2019; 25(6): 890-908. https://doi.org/10.1089/mdr.2018.0319.
2. Lozano C, Torres C. Actualización en la resistencia antibiótica en Gram positivos [Update on antibiotic resistance in Gram-positive bacteria]. Enferm Infecc Microbiol Clin 2017; 35 Suppl 1:2-8. https://doi.org/10.1016/S0213-005X(17)30028-9.
3. Titov I, Wunderink RG, Roquilly A, Rodríguez Gonzalez D, David-Wang A, Boucher HW, et al. A Randomized, Double-blind, Multicenter Trial Comparing Efficacy and Safety of Imipenem/Cilastatin/Relebactam Versus Piperacillin/Tazobactam in Adults With Hospital-acquired or Ventilator-associated Bacterial Pneumonia (RESTORE-IMI 2 Study). Clin Infect Dis 2021; 73(11): e4539-e4548. https://doi.org/10.1093/cid/ciaa803.
4. Campanella TA, Gallagher JC. A Clinical Review and Critical Evaluation of Imipenem-Relebactam: Evidence to Date. Infect Drug Resist 2020; 13: 4297-4308. https://doi.org/10.2147/IDR.S224228.
5. Mushtaq S, Meunier D, Vickers A, Woodford N, Livermore DM. Activity of imipenem/relebactam against Pseudomonas aeruginosa producing ESBLs and carbapenemases. J Antimicrob Chemother 2021; 76(2): 434-442. https://doi.org/10.1093/jac/dkaa456.
6. Cruz-López F, Martínez-Meléndez A, Morfin-Otero R, Rodriguez-Noriega E, Maldonado-Garza HJ, Garza-González E. Efficacy and In Vitro Activity of Novel Antibiotics for Infections With Carbapenem-Resistant Gram-Negative Pathogens. Front Cell Infect Microbiol 2022; 12: 884365. https://doi.org/10.3389/fcimb.2022.884365.
7. Lapuebla A, Abdallah M, Olafisoye O, Cortes C, Urban C, Landman D, et al. Activity of Imipenem with Relebactam against Gram-Negative Pathogens from New York City. Antimicrob Agents Chemother 2015; 59(8): 5029-31. https://doi.org/ 10.1128/AAC.00830-15.
8. Yang T-Y, Hsieh Y-J, Kao L-T, Liu GH, Lian S-H, Wang L-C, et al. Activities of imipenem-relebactam combination against carbapenem-nonsusceptible Enterobacteriaceae in Taiwan. J Microbiol Immunol Infect 2022; 55(1): 86-94. https://doi.org/ 10.1016/j.jmii.2021.02.001.
9. Heo YA. Imipenem/Cilastatin/Relebactam: A Review in Gram-Negative Bacterial Infections. Drugs 2021; 81(3): 377-388. https://doi.org/10.1007/s40265-021-01471-8.
10. Kurihara D, Matsumoto S, Kishi N, Ishii Y, Mori M. In Vitro Antibacterial Activity of Imipenem/Relebactam against Clinical Isolates in Japan. Microbiol Spectr 2022; 10(2): e0223521. https://doi.org/10.1128/spectrum.02235-21.
11. Gebre AB, Begashaw TA, Ormago MD. Bacterial profile and drug susceptibility among adult patients with community acquired lower respiratory tract infection at tertiary hospital, Southern Ethiopia. BMC Infect Dis 2021; 21(1): 440. https://doi.org/10.1186/s12879-021-06151-2.
12. Lob SH, Karlowsky JA, Young K, Motyl MR, Hawser S, Kothari ND, et al. In vitro activity of imipenem-relebactam against resistant phenotypes of Enterobacteriaceae and Pseudomonas aeruginosa isolated from intraabdominal and urinary tract infection samples - SMART Surveillance Europe 2015-2017. J Med Microbiol 2020; 69(2): 207-217. https://doi.org/10.1099/jmm.0.001142.
13. Mansour H, Ouweini AEL, Chahine EB, Karaoui LR. Imipenem/cilastatin/relebactam: A new carbapenem β-lactamase inhibitor combination. Am J Health Syst Pharm 2021; 78(8): 674-683. https://doi.org/10.1093/ajhp/zxab012.
14. Dhingra S, Rahman NAA, Peile E, Rahman M, Sartelli M, Hassali MA, et al. Microbial Resistance Movements: An Overview of Global Public Health Threats Posed by Antimicrobial Resistance, and How Best to Counter. Front Public Health 2020; 8: 535668. https://doi.org/10.3389/fpubh.2020.535668.
15. Papp-Wallace KM, Barnes MD, Alsop J, Taracila MA, Bethel CR, Becka SA, et al. Relebactam Is a Potent Inhibitor of the KPC-2 β-Lactamase and Restores Imipenem Susceptibility in KPC-

Producing Enterobacteriaceae. Antimicrob Agents Chemother 2018; 62(6): e00174-18. https://doi.org/10.1128/AAC.00174-18.

16. O'Donnell JN, Lodise TP. New Perspectives on Antimicrobial Agents: Imipenem-Relebactam. Antimicrob Agents Chemother 2022; 66(7): e0025622. https://doi.org/10.1128/aac.00256-22.
17. Zhanel GG, Lawrence CK, Adam H, Schweizer F, Zelenitsky S, Zhanel M, et al. Imipenem-Relebactam and Meropenem-Vaborbactam: Two Novel Carbapenem-β-Lactamase Inhibitor Combinations. Drugs 2018; 78(1): 65-98. https://doi.org/10.1007/s40265-017-0851-9.
18. Salmon-Rousseau A, Martins C, Blot M, Buisson M, Mahy S, Chavanet P, et al. Comparative review of imipenem/cilastatin versus meropenem. Med Mal Infect 2020; 50(4): 316-322. https://doi.org/10.1016/j.medmal.2020.01.001