Medical Laboratory Technology Journal
11(1), 2025, 72-81
Received 2025-22-04; Revised 2025-05-05; Accepted 2025-22-05
Available online at : http://ejurnal-analiskesehatan.web.id

Amino Acid Mutations of OprD Protein in *Pseudomonas aeruginosa* After Meropenem Exposure

***Agus Evendi, Tiara Dini Harlita, Sresta Azahra**

[1]Department of Medical Laboratory Technology, Poltekkes Kemenkes Kalimantan Timur, Indonesia. *E-mail: agusevendi.biomed@gmail.com
DOI: 10.31964/mltj.v11i1.646

Abstract: *Pseudomonas aeruginosa* is a gram-negative pathogen associated with nosocomial infections and increased resistance to carbapenems, often linked to porin OprD inactivation. This study aimed to analyse amino acid substitutions in the OprD protein of two meropenem-sensitive *Pseudomonas aeruginosa* isolates (AK36 and AK237b) after 12 days of in vitro exposure to subinhibitory meropenem concentration (0.5 µg/mL). DNA was extracted at three time points (days 0, 5, and 12) and the *oprD* gene was sequenced using Sanger sequencing. Protein sequences were aligned and modelled using Swiss-Model to identify mutations and to assess structural changes. By day 12, AK36 had Gln67Lys and Gly68Ser substitutions, whereas AK237b had Glu169Lys. Structural modelling suggests these mutations may alter porin conformation and reduce membrane permeability. Despite no increase in the MIC, oprD expression was suppressed, indicating early adaptation. These findings support the hypothesis that prolonged meropenem pressure induces molecular changes that precede phenotypic resistance. This study highlights the importance of monitoring porin mutations as an early indicator of carbapenem resistance in clinical microbiology. This could help to improve antibiotic stewardship by identifying isolates at risk of developing resistance before it becomes clinically apparent.
Keywords: *Pseudomonas aeruginosa*; carbapenem resistance; OprD mutation; meropenem exposure.

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic gram-negative bacterium that has emerged as a significant etiological agent of nosocomial infections globally. This organism presents a substantial clinical threat, particularly to immunocompromised individuals, including those undergoing chemotherapy, transplantation, or extended stays in intensive care units (ICUs). Its metabolic adaptability, ability to form biofilms, and inherent resistance mechanisms have contributed to its success as a pathogen in healthcare environments. A particularly concerning characteristic of *Pseudomonas aeruginosa* is its intrinsic and acquired resistance to a broad spectrum of antibiotics, including β-lactams, aminoglycosides, and fluoroquinolones. Its resistance to carbapenems is particularly concerning, a class of β-lactam antibiotics often considered a last resort for treating infections caused by multidrug-resistant gram-negative bacteria. Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) has been associated with high morbidity and mortality, especially in vulnerable patients, due to delayed effective therapy and limited treatment options (Bassetti et al., 2018).

The mechanisms responsible for carbapenem resistance in *Pseudomonas aeruginosa* are complex and multifaceted. These mechanisms encompass the production of carbapenemases, the overexpression of efflux pumps, the upregulation of AmpC β-lactamases, and notably, the loss or modification of outer membrane

Corresponding Author: Agus Evendi
Department of Medical Laboratory Technology, Poltekkes Kemenkes Kalimantan Timur
Jalan Kurnia Makmur No. 64 Loa Janan Ilir Samarinda Kalimantan Timur 75131, Indonesia.
Email: agusevendi.biomed@gmail.com

porins. Alterations in the OprD porin protein are widely acknowledged as a pivotal mechanism in isolates that do not produce carbapenemases. OprD is a substrate-specific porin that facilitates the uptake of basic amino acids and certain antibiotics, including meropenem and imipenem. Its downregulation or inactivation decreases outer membrane permeability by limiting antibiotic entry into bacterial cells and contributing to resistance. Blair et al. (2014) and Yano et al. (2024) highlighted that the downregulation of *oprD* expression and structural mutations in the *oprD* gene constitute adaptive strategies employed by *Pseudomonas aeruginosa* in response to antibiotic pressure. These strategies frequently coincide with enhanced activity of efflux systems.

Epp et al. (2001) and Hujer et al. (2022) demonstrated that structural modifications in the C-terminal domain of OprD, particularly within loop L7, significantly affect meropenem permeability. Their findings underscore that structural changes in OprD can influence susceptibility to carbapenems even without substantial efflux activity. Similarly, Ocampo-Sosa et al. (2012) and Zhao et al. (2023) reported that premature stop codons and frameshift mutations in *oprD* are prevalent in both phenotypically resistant and susceptible clinical isolates, suggesting that such mutations may precede phenotypic resistance and serve as early adaptive mechanisms.

Lee and Ko (2012) and Teo et al. (2021) corroborated these findings by demonstrating that downregulation of *oprD* was evident even in isolates that did not exhibit elevated minimum inhibitory concentrations (MICs) to carbapenems. Qin et al. (2022) further elucidated that mutations affecting the loop structure and channel conformation of OprD resulted in diminished meropenem accumulation within the periplasmic space, thereby impairing the drug's bactericidal efficacy. Kao et al. (2016) and Ng et al. (2021) identified that structural mutations, including truncation of loop L7 and the presence of stop codons, were predominant mechanisms underlying imipenem resistance among clinical isolates in Taiwan. These structural impairments adversely affect porin selectivity and transport capacity, crucial for meropenem uptake.

Glen et al. (2021), Kim et al. (2024), and Teo et al. (2021) have underscored the significance of OprD as a pivotal entry point for carbapenems, elucidating that mutations leading to conformational alterations or complete loss of expression frequently result in carbapenem resistance, even in the absence of enzymatic degradation. Poole (2011), Teo et al. (2021), and Stanton et al. (2022) characterized *Pseudomonas aeruginosa* resistance as a complex process, with *oprD* inactivation serving as a crucial element of non-carbapenemase-mediated resistance. Additionally, this mechanism often operates in conjunction with the derepression of *ampC* or the upregulation of efflux systems, such as MexAB-OprM, thereby augmenting the resistance phenotype.

Fuhs et al. (2024) underscored the critical role of combined resistance mechanisms, noting that the inactivation of *oprD*, in conjunction with efflux hyperproduction and β-lactamase expression, constitutes a formidable triad of resistance. Notably, they demonstrated that resistance can persist even in the absence of carbapenemase genes, highlighting the significance of porin-mediated pathways. Regulatory elements such as MexT and AmpR, which are modulated by environmental factors including oxidative stress and the presence of heavy metals, can indirectly influence *oprD* expression, as emphasized by Lister et al. (2009), Bisht et al. (2021), Liu et al. (2022), and Da Cruz Nizer et al. (2021).

Wu et al. (2024) observed that diminished *oprD* function is frequently associated with mutations within its open reading frame (ORF) or promoter regions, or

through regulatory repression. Their study found such alterations to be prevalent in isolates demonstrating resistance, despite the absence of carbapenemase genes. Castanheira et al. (2023) further reported that the overexpression of *ampC* and MexXY efflux pumps significantly elevated the minimum inhibitory concentrations (MICs) for meropenem, particularly when combined with structural disruption of OprD. Additionally, Wang et al. (2025) provided a novel insight by identifying isolates that neither produced carbapenemase nor exhibited active efflux, yet displayed resistance solely due to *oprD* inactivation. Importantly, restoring *oprD* expression in these isolates reversed the resistant phenotype, underscoring the critical role of this protein.

Evendi et al. (2024) previously reported a significant downregulation of *oprD* expression following 12 days of meropenem exposure in vitro, which was associated with an increased MIC, despite the stable expression of *ampC* and *mexA*. These findings were observed across a broader set of isolates, indicating that, in certain instances, the suppression of *oprD* alone may result in an increased MIC, contingent upon the genetic background of the isolate. Conversely, the present study concentrated on two specific isolates, AK36 and AK237b, both of which demonstrated structural mutations and transcriptional suppression of *oprD*, yet did not exhibit any increase in MIC. This contrast underscores that molecular adaptations may precede phenotypic resistance and are potentially influenced by isolate-specific factors such as baseline membrane permeability, efflux capacity, or compensatory pathways. Despite the expanding body of literature on the role of OprD in carbapenem resistance, most studies have concentrated on gene expression, enzymatic activity, or resistance phenotypes. Research examining the temporal dynamics of amino acid substitutions in OprD in response to controlled in vitro antibiotic exposure remains limited. Moreover, detailed structural modelling does not elucidate how specific amino acid changes alter porin function.

In this study, we aimed to examine the alterations in the amino acid sequence of the OprD protein in two meropenem-sensitive *Pseudomonas aeruginosa* isolates following 12 days of in vitro exposure to sub-inhibitory concentrations of meropenem. The objective was to identify specific amino acid substitutions and evaluate their potential effects on the structure and function of OprD through in silico 3D protein modelling. This research offers novel insights into the early molecular events under antibiotic pressure that can facilitate bacterial adaptation, potentially leading to phenotypic resistance. By concentrating on the structural implications of these substitutions, this study enhances the understanding of resistance evolution in *Pseudomonas aeruginosa* and highlights the significance of molecular surveillance in antibiotic stewardship.

MATERIALS AND METHODS

This in vitro experimental study, conducted in the Clinical Microbiology Department at the Faculty of Medicine, Universitas Indonesia, spanned from August 2020 to August 2023. The primary objective was to examine alterations in the amino acid sequence of the OprD protein in two *Pseudomonas aeruginosa* isolates (AK36 and AK237b) following exposure to meropenem. These two isolates were selected because they were initially susceptible to meropenem (MIC ≤ 0,25 µg/mL) and demonstrated distinct amino acid substitutions in OprD after 12 days of exposure, making them suitable for evaluating early molecular changes in porin-mediated resistance. Ethical approval was secured from the Faculty of Medicine Ethics Committee, Universitas Indonesia (Approval Nomor KET 273/UN2.F1/ETIK/PPM.00.02/2020).

Both isolates were verified as sensitive to meropenem and cultured in Mueller-Hinton Broth (Oxoid). A subinhibitory concentration of 0.5 µg/mL meropenem (Merck) was employed over a 12-day exposure period, with daily transfers to fresh medium containing meropenem. Ten biological replicates were prepared in separate screw-capped tubes to ensure experimental reliability. Sampling occurred on Days 0, 5, and 12.

Genomic DNA extraction was performed using the QIAamp® DNA Mini Kit (QIAGEN), and the oprD gene was amplified via PCR with specific primers. Each PCR reaction was performed in a 25 µL volume containing a final primer concentration of 0.2 µM. The thermal cycling conditions included an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 7 min. Amplicons were verified through 1.5% agarose gel electrophoresis and sequenced bidirectionally using the Sanger method (1st BASE, Singapore). The resulting DNA sequences were translated into amino acid sequences using BioEdit software and aligned with the *Pseudomonas aeruginosa* PAO1 reference strain.

To evaluate the impact of mutations on protein conformation, the 3D structure of OprD was modelled using the Swiss-Model server. Comparisons between the wild-type (day 0) and mutated (day 12) protein models were conducted to identify potential structural changes in the porin channel that could affect meropenem permeability.

RESULTS AND DISCUSSION

The analysis of the amino acid sequence of the OprD protein in two *Pseudomonas aeruginosa* isolates, AK36 and AK237b, identified mutations following 12 days of exposure to meropenem. No mutations were observed on days 0 or 5 in either isolate. However, by day 12, isolate AK36 exhibited two amino acid substitutions: glutamine (Gln) at position 67 was replaced by lysine (Lys), and glycine (Gly) at position 68 was replaced by serine (Ser). Conversely, isolate AK237b demonstrated a single substitution at position 169, where glutamic acid (Glu) was replaced by lysine (Lys).

Three-dimensional structural modelling using Swiss-Model suggested that these amino acid substitutions may alter porin conformation. In AK36, the Gln67Lys and Gly68Ser mutations are situated in the external loop of OprD, a region implicated in substrate selectivity. The transition from a neutral to a positively charged residue, particularly from Gln to Lys, may disrupt electrostatic interactions within the channel. The Gly68Ser substitution introduces a polar side chain, potentially affecting loop flexibility and alignment. In AK237b, the Glu169Lys mutation is located deeper within the channel in the β-barrel domain, possibly influencing the meropenem entry pathway. A summary of the identified *oprD* mutations, their structural locations, effect on protein structure, gene expression levels, and MIC values is presented in Table 1.

These findings support the hypothesis that prolonged exposure to sub-inhibitory concentrations of meropenem can induce structural adaptations in the OprD protein. Notably, despite mutations, both isolates remained phenotypically sensitive to meropenem, as indicated by MIC measurements (≤ 0.25 µg/mL). This suggests that such changes may represent early-stage adaptations preceding overt resistance.

Table 1. Summary of *oprD* Mutations, Structural Implication, Gene Expression, and MIC in *Pseudomonas aeruginosa* Isolates after Meropenem Exposure

Isolate	Mutation(s)	Position in OprD	Structural Implication	*oprD* Expression (Day 12)	MIC (µg/mL)
AK36	Gln67Lys, Gly68Ser	External loop (Loop L7)	Alter electrostatic charge and flexibility	0.44	≤0.25
AK237b	Glu169Lys	B-barrel domain (internal channel)	May affect meropenem entry pathway	0.09	≤0.25

Gln=Glutamine, Lys=Lysine, Gly=Glycine, Ser=Serine, Glu=Glutamic Acid

Gene expression analysis conducted via real-time RT-PCR revealed a decrease in *oprD* expression over time in both isolates. Notably, the isolate AK237b significantly reduced from 0.28 (day 5) to 0.09 (day 12). In AK36, *oprD* expression remained consistently low (0.38 to 0.44) despite no change in MIC. The significant correlation between MIC and *oprD* expression on day 12 ($r = -0.80$, $p = 0.01$) indicates that oprD suppression is critical in modulating susceptibility.

These findings align with the results of Epp et al. (2001), who demonstrated that mutations in loop L7 of OprD can modify porin permeability without influencing MexEF-OprN expression. Similarly, Ocampo-Sosa et al. (2012) and Lee and Ko (2012) observed that structural alterations in OprD can precede phenotypic resistance, serving as early indicators. This supports the notion that mutation or inactivation of OprD is a primary mechanism in non-carbapenemase-mediated resistance.

In the case of AK36, a combined structural alteration (Gln67Lys, Gly68Ser) and overexpression (from 3.18 to 3.61-fold) were observed, while *oprD* remained suppressed. This synergy between reduced porin influx and increased efflux is consistent with the mechanisms reported by Kao et al. (2016), Poole (2011), and Qin et al. (2022). Glen et al. (2021) also emphasized that diminished OprD function, even in the absence of MIC elevation, restricts the intracellular accumulation of meropenem, enabling bacteria to adapt silently under antibiotic stress.

Conversely, AK237b increased *ampC* expression (from 2.33 to 2.51), suggesting that alongside the *oprD* mutation (Glu169Lys), β-lactamase activity may contribute to resistance priming. Although MICs did not exceed the susceptibility breakpoint, the combination of porin alteration and *ampC* upregulation reflects the findings of Castanheira et al. (2023) and Wang et al. (2025), who noted that resistance can develop solely through OprD disruption without the involvement of efflux or carbapenemase genes.

Figure 1 illustrates the predicted 3D structural changes in OprD for AK36 on day 12, indicating potential narrowing of the pores. Similarly, Figure 2 presents the altered conformation of AK237b, demonstrating the deeper impact of the Glu169Lys substitution in the channel.

Although these structural models provide valuable insights, it is important to note the limitations of *in silico* 3D modelling. The Swiss-Model predictions used in this study were based on homology templates and static conformations, which may not fully capture the dynamic behaviour of membrane proteins under physiological conditions. Moreover, these models do not account for post-translational modifications

or interactions with other membrane components that may influence porin function in vivo. Experimental validation using crystallographic data or functional assays is required to confirm the structural impacts inferred from modelling. The perspective articulated by Motta et al. (2020) underscored the complementary function of proteomic and experimental methodologies in conjunction with computational techniques for characterizing membrane-associated proteins, such as OprD.

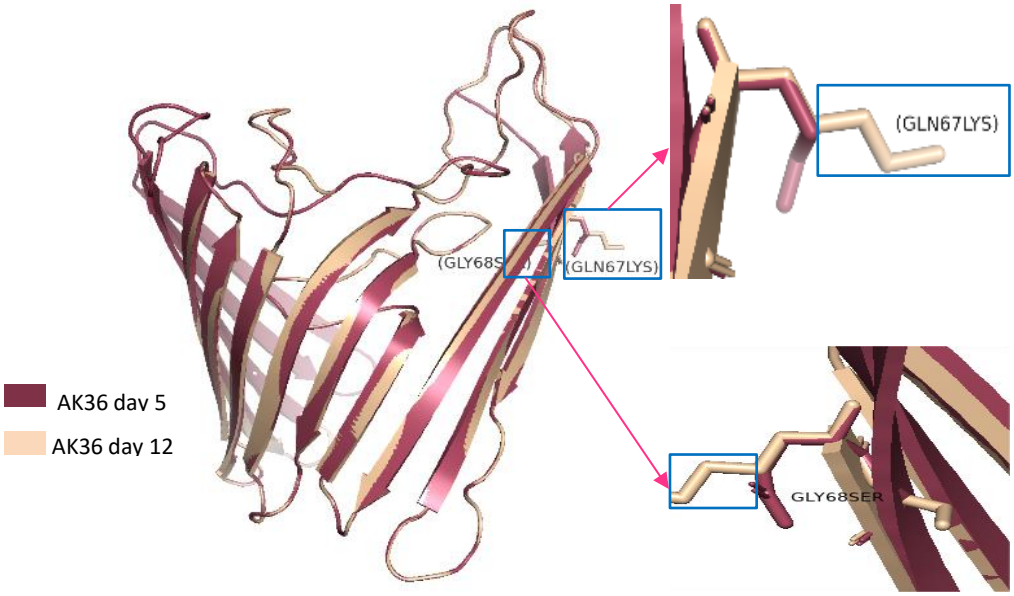

Figure 1. Predicted 3D structure of the OprD protein in isolate AK36 after 12 days of meropenem exposure

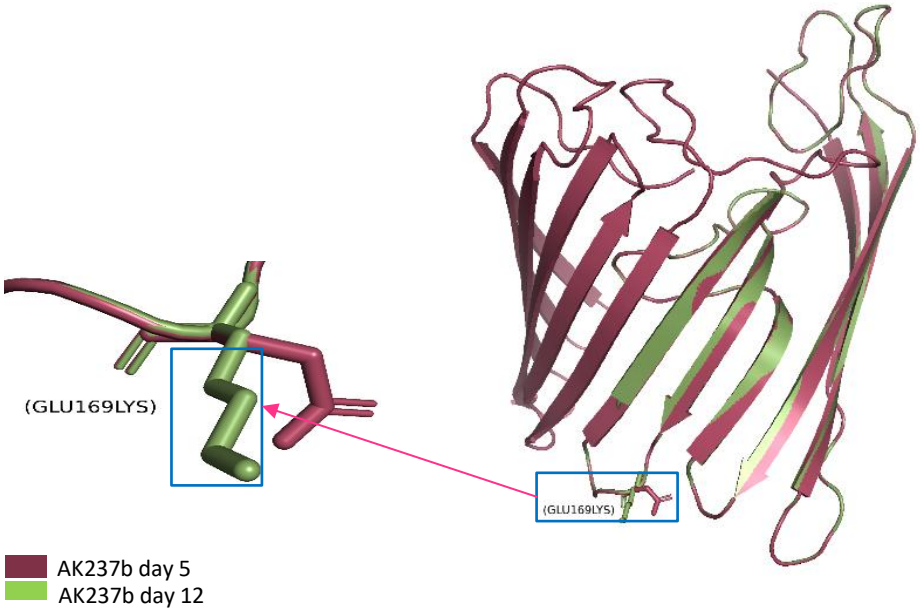

Figure 2. Predicted 3D structure of the OprD protein in isolate AK237b after 12 days of meropenem exposure

Although no significant increase in MICs was observed, structural and transcriptional changes highlighted a crucial adaptation phase. As supported by Wu et al. (2024), such mutations or regulatory suppression of OprD are early steps in the

evolution of resistance. The mutations observed, particularly Gln67Lys and Gly68Ser, alter the charge and polarity at critical sites, potentially reducing carbapenem affinity. Furthermore, Fuhs et al. (2024) posited that the disruption of OprD frequently coexists with regulatory mutations in mexR or ampR, thereby facilitating a gradual increase in resistance. Our findings corroborate this notion, demonstrating that resistance may develop incrementally before it becomes phenotypically apparent. Blair et al. (2014) also suggested that the loss of OprD and increased efflux expression are central to resistance in Gram-negative bacteria lacking carbapenemase genes. Notably, susceptibility was maintained even with combinations such as *ampC* overexpression and *oprD* mutation (AK237b) or *mexA* overexpression and *oprD* mutation (AK36). This suggests that, while these mutations contribute to resistance potential, a certain threshold must be surpassed before establishing phenotypic resistance. The absence of MIC elevation in this study may be because the structural mutations in OprD, although present, have not yet caused sufficient disruption to impair meropenem uptake significantly.

Additionally, efflux or β-lactamase mechanisms alone may not have reached the functional level required to manifest phenotypic resistance. As Poole (2011) highlighted, cumulative mechanisms must exceed a functional tipping point to affect clinical outcomes. These results underscore the importance of early molecular detection of resistance mechanisms, even in phenotypically sensitive strains. Castanheira et al. (2023) noted that routine surveillance focusing solely on MICs may overlook the adaptive genetic changes that presage future resistance. This study contributes to a growing body of evidence that structural and regulatory OprD alterations are early indicators of reduced carbapenem susceptibility. The limitations of this study include the small sample size and the focus on only two isolates for mutation analysis. Functional validation using protein expression or permeability assays has not been performed. Additionally, only three resistance genes (*ampC*, *mexA*, and *oprD*) were analyzed, excluding other potential contributors such as regulatory systems (*mexR*, *ampR*) or acquired genes. Further studies with larger isolate collections, functional assays, and comprehensive genomic profiling are necessary to determine the predictive value of these mutations in the clinical setting.

CONCLUSION

In vitro exposure of sensitive *Pseudomonas aeruginosa* isolates to meropenem over 12 days resulted in specific amino acid substitutions within the OprD protein. The mutations Gln67Lys and Gly68Ser in AK36 and Glu169Lys in AK237b emerged following sustained antibiotic pressure. Structural modelling indicates that these mutations may alter porin conformation, suggesting an initial adaptive mechanism that could impede meropenem transport. These findings underscore the significance of molecular monitoring in predicting resistance development, even when clinical susceptibility remains unchanged.

ACKNOWLEDGMENTS

The authors would like to thank and express their appreciation to the Director of Poltekkes Kemenkes Kalimantan Timur, who supported this research.

CONFLICT OF INTEREST

The authors declare no conflict of interest for this research and publication.

REFERENCES

Bassetti, M., Croxatto, A., Guery, B., Russo, A., Vena, A., & Calandra, T. (2018). Rational approach in the management of *Pseudomonas aeruginosa* infections. *Current Opinion in Infectious Diseases, 31*(6), 578–586. https://doi.org/10.1097/qco.0000000000000505

Bisht, K., Wakeman, C. A., Skaar, E. P., Moore, J. L., & Caprioli, R. M. (2021). Impact of temperature-dependent phage expression on *Pseudomonas aeruginosa* biofilm formation. *NPJ Biofilms and Microbiomes, 7*(1). https://doi.org/10.1038/s41522-021-00194-8

Blair, J. M. A., Baylay, A. J., Piddock, L. J. V., Ogbolu, D. O., & Webber, M. A. (2014). Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology, 13*(1), 42–51. https://doi.org/10.1038/nrmicro3380

Castanheira, M., Hubler, C. M., Doyle, T. B., Devries, S., & Shortridge, D. (2023). Vaborbactam increases meropenem susceptibility in *Pseudomonas aeruginosa* clinical isolates displaying MexXY and AmpC upregulation. *mSphere, 8*(5). https://doi.org/10.1128/msphere.00162-23

Da Cruz Nizer, W. S., Inkovskiy, V., Cassol, E., Strempel, N., Versey, Z., & Overhage, J. (2021). Oxidative stress response in *Pseudomonas aeruginosa*. *Pathogens, 10*(9), 1187. https://doi.org/10.3390/pathogens10091187

Epp, S. F., Köhler, T., Frey, J., Plésiat, P., Michéa-Hamzehpour, M., & Pechère, J.-C. (2001). C-terminal region of *Pseudomonas aeruginosa* outer membrane porin OprD modulates susceptibility to meropenem. *Antimicrobial Agents and Chemotherapy, 45*(6), 1780–1787. https://doi.org/10.1128/aac.45.6.1780-1787.2001

Evendi, A., Karuniawati, A., Ibrahim, F., & Asmarinah, A. (2024). Genetic and phenotypic of *Pseudomonas aeruginosa* sensitive to meropenem antibiotics after exposure to meropenem. *Iranian Journal of Microbiology, 16*(3). https://doi.org/10.18502/ijm.v16i3.15760.

Fuhs, D. T., Rogers, K. E., Tait, J. R., Oliver, A., López-Causapé, C., Nation, R. L., Lee, W. L., Shackleford, D. M., Landersdorfer, C. B., & Cortés-Lara, S. (2024). Elucidating effects of single and multiple resistance mechanisms on bacterial response to meropenem by quantitative and systems pharmacology modeling and population genomics. *Cold Spring Harbor Laboratory*. https://doi.org/10.1101/2024.02.17.579784

Glen, K. A., & Lamont, I. L. (2021). β-lactam resistance in *Pseudomonas aeruginosa*: Current status, future prospects. *Pathogens, 10*(12), 1638. https://doi.org/10.3390/pathogens10121638

Hujer, A. M., Winkler, M. L., Bonomo, R. A., Domitrovic, T. N., Abdelhamed, A. M., Painter, R. E., Mack, A. R., Jacobs, M. R., Bethel, C. R., Clarke, T., D'Souza, R., Young, K., White, R. C., Taracila, M. A., Watkins, R. R., Fouts, D. E., Rojas, L. J., & Marshall, S. H. (2022). Imipenem/relebactam resistance in clinical isolates of extensively drug resistant *Pseudomonas aeruginosa*: Inhibitor-resistant β-lactamases and their increasing importance. *Antimicrobial Agents and Chemotherapy, 66*(5). https://doi.org/10.1128/aac.01790-21

Kao, C.-Y., Chen, S.-S., Yan, J.-J., Wu, H.-M., Hung, K.-H., Hsueh, P.-R., & Wu, J.-J. (2016). Overproduction of active efflux pump and variations of OprD dominate in imipenem-resistant *Pseudomonas aeruginosa* isolated from patients with bloodstream infections in Taiwan. *BMC Microbiology, 16*(1). https://doi.org/10.1186/s12866-016-0719-2

Kim, N., Lee, D. E., Kim, Y. K., Lee, J. C., Park, S. Y., Kwon, K. T., Ko, S. Y., Lee, J. C., Kim, Y. K., Kwon, K. T., & Kim, S. Y. (2024). Clonal distribution and its association with the carbapenem resistance mechanisms of carbapenem-non-susceptible *Pseudomonas aeruginosa* isolates from Korean Hospitals. *Annals of Laboratory Medicine*, *44*(5), 410–417. https://doi.org/10.3343/alm.2023.0369

Lee, J.-Y., & Ko, K. S. (2012). OprD mutations and inactivation, expression of efflux pumps and AmpC, and metallo-β-lactamases in carbapenem-resistant *Pseudomonas aeruginosa* isolates from South Korea. *International Journal of Antimicrobial Agents, 40*(2), 168–172. https://doi.org/10.1016/j.ijantimicag.2012.04.004

Lister, P. D., Wolter, D. J., & Hanson, N. D. (2009). Antibacterial-resistant *Pseudomonas aeruginosa*: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clinical Microbiology Reviews, 22*(4), 582–610. https://doi.org/10.1128/cmr.00040-09

Liu, Z., Chen, S., Li, T., Huang, J., Xu, Z., Xu, Z., Zhang, L.-H., & Duan, C. (2022). CzcR Is Essential for Swimming Motility in *Pseudomonas aeruginosa* during Zinc Stress. *Microbiology Spectrum, 10*(6). https://doi.org/10.1128/spectrum.02846-22

Motta, S., Vecchietti, D., Di Silvestre, D., Polissi, A., Mauri, P., Bertoni, G., Brunetti, P., & Martorana, A. M. (2020). The landscape of *Pseudomonas aeruginosa* membrane-associated proteins. *Cells*, *9*(11), 2421. https://doi.org/10.3390/cells9112421

Ng, M. Y., Li, H., Ghelfi, M. D., Goldman, Y. E., & Cooperman, B. S. (2021). Ataluren and aminoglycosides stimulate read-through of nonsense codons by orthogonal mechanisms. *Proceedings of the National Academy of Sciences*, *118*(2). https://doi.org/10.1073/pnas.2020599118

Ocampo-Sosa, A. A., Oliver, A., Domínguez, M. A., Macia, M. D., Peña, C., Cabot, G., Martínez-Martínez, L., Zamorano, L., Moncalián, G., Roman, E., Rodríguez, C., Tubau, F., Suárez, C., & Moya, B. (2012). Alterations of OprD in carbapenem-intermediate and -susceptible strains of *Pseudomonas aeruginosa* isolated from patients with bacteremia in a Spanish multicenter study. *Antimicrobial Agents and Chemotherapy, 56*(4), 1703–1713. https://doi.org/10.1128/aac.05451-11

Poole, K. (2011). Pseudomonas Aeruginosa: Resistance to the Max. *Frontiers in Microbiology, 2*(Suppl 1). https://doi.org/10.3389/fmicb.2011.00065

Qin, S., Xiao, W., Zhou, C., Pu, Q., Deng, X., Lan, L., Liang, H., Song, X., & Wu, M. (2022). *Pseudomonas aeruginosa*: pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. *Signal Transduction and Targeted Therapy, 7*(1). https://doi.org/10.1038/s41392-022-01056-1

Stanton, R. A., Schutz, K., Mounsey, J., Halpin, A. L., Lynfield, R., Campbell, D., Dumyati, G., Li, L., Cassidy, P. M., Adamczyk, M., Breaker, E., Lutgring, J. D., Vaeth, E., Hancock, E. B., Karlsson, M., Grass, J. E., Tsay, R., Walters, M. S., Vagnone, P., … Bulens, S. (2022). Whole-genome sequencing reveals diversity of carbapenem-resistant *Pseudomonas aeruginosa* collected through CDC's emerging infections program, United States, 2016-2018. *Antimicrobial Agents and Chemotherapy, 66*(9). https://doi.org/10.1128/ aac.00496-22

Teo, J. Q.-M., Tang, C. Y., Lim, J. C., Lee, S. J.-Y., Tan, S. H., Koh, T.-H., Sim, J. H.-C., Tan, T.-T., Kwa, A. L.-H., & Ong, R. T.-H. (2021). Genomic characterization

of carbapenem-non-susceptible *Pseudomonas aeruginosa* in Singapore. *Emerging Microbes & Infections, 10*(1), 1706–1716. https://doi.org/10.1080/22221751.2021.1968318

Wang, M., Zhang, Y., Pei, F., Liu, Y., & Zheng, Y. (2025). Loss of OprD function is sufficient for carbapenem-resistance-only but insufficient for multidrug resistance in *Pseudomonas aeruginosa*. *BMC Microbiology, 25*(1). https://doi.org/10.1186/s12866-025-03935-3

Wu, W., Huang, J., & Xu, Z. (2024). Antibiotic influx and efflux in *Pseudomonas aeruginosa*: Regulation and therapeutic implications. *Microbial Biotechnology, 17*(5). https://doi.org/10.1111/1751-7915.14487

Yano, H., Anzai, E., Yahara, K., Zuo, H., Aoki, S., Sugawara, Y., Hirabayashi, A., Kayama, S., Hayashi, W., Sugai, M., Kajihara, T., Kitamura, N., Kawakami, S., & Tamma, P. D. (2024). Nationwide genome surveillance of carbapenem-resistant *Pseudomonas aeruginosa* in Japan. *Antimicrobial Agents and Chemotherapy, 68*(5). https://doi.org/10.1128/aac.01669-23

Zhao, Y., Wang, J., Guo, J., Jelsbak, L., Chen, S., Chen, D., Chen, E., Xie, L., Chen, K., Chan, E. W. C., Xie, M., & Chen, W. (2023). Epidemiological and genetic characteristics of clinical carbapenem-resistant *Pseudomonas aeruginosa* strains in Guangdong Province, China. *Microbiology Spectrum, 11*(3). https://doi.org/10.1128/spectrum.04261-22