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SHORT COMMUNICATION

Susceptibility of ceftolozane/tazobactam against multidrug-resistant and carbapenem-resistant *Pseudomonas aeruginosa*

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SUMMARY

Ceftolozane (CTLZ) is a novel cephalosporin antibiotic that exhibits broad-spectrum activity against gram-negative pathogens, including *Pseudomonas aeruginosa,* especially when combined with tazobactam (TAZ). We examined the minimum inhibitory concentration (MIC) of CTLZ/TAZ for 21 multidrug-resistant *P. aeruginosa* (MDRP) and eight carbapenem-resistant *P. aeruginosa* (CRPA) strains isolated at Okayama University Hospital, Japan. Consequently, 81% (17/21) of the MDRP strains and 25% (2/8) of the CRPA strains were resistant to CTLZ/TAZ (MIC >8 μg/mL). All 18 bla_{IMP}-positive strains showed resistance to CTLZ/TAZ, whereas the drug retained *in vitro* susceptibility in 54.5% (6/11 strains) of bla_{IMP}-negative strains.

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The World Health Organization (WHO) recognizes the emerging threat of antimicrobial resistance (AMR) as one of the top ten global public health concerns. A recent estimate suggested that deaths resulting from AMR would reach 10 million by 2050 if no action is taken. In this context, the global spread of AMR demands the development of new therapeutic strategies and the promotion of antimicrobial stewardship.

Ceftolozane/tazobactam (CTLZ/TAZ) is a novel antimicrobial drug combination comprising a newly-developed anti-pseudomonal cephalosporin and a conventional β-lactamase inhibitor. CTLZ/TAZ is effective *in vitro* against gram-negative pathogens, including *Pseudomonas aeruginosa*, but lacks potency against carbapenemase-producing strains (Karlowsky, 2021). Among the variety of carbapenemases with distinct genotypic and phenotypic characteristics, bla_{IMP} is predominant in Japan (Nakayama, 2022). Previous studies have suggested that bla_{IMP}-positive *P. aeruginosa* strains are resistant to CTLZ/TAZ (Vallabhaneni, 2021). However, owing to the limited number of isolates tested, the clinical efficacy of CTLZ/TAZ against bla_{IMP}-positive *P. aeruginosa* is yet to be ascertained in Japan. Here, we investigated the antimicrobial susceptibility of multidrug-resistant *P. aeruginosa* (MDRP) and carbapenem-resistant *P. aeruginosa* (CRPA) to CTLZ/TAZ.

We used MDRP and CRPA strains isolated in our clinical microbiology laboratory between 2009 and 2020 and between 2017 and 2019, respectively. We defined MDRP as *P. aeruginosa* strains showing resistance to imipenem (minimum inhibitory concentration [MIC] ≥16 μg/mL), ciprofloxacin (MIC ≥4 μg/mL), and amikacin (MIC ≥32 μg/mL). In addition, we defined CRPA as *P. aeruginosa* strains resistant to both imipenem (MIC ≥16 μg/mL) and meropenem (MIC ≥16 μg/mL). MICs, including those for CTLZ/TAZ, were measured by the broth microdilution method, using Dry Plate 'Eiken' (Eiken Chemical Co., Ltd, Tokyo, Japan), based on the Clinical and Laboratory Standards Institute (CLSI) M100, 31st edition. Both MDRP and CRPA were examined using the sodium mercapto acetate (SMA) test for metallo-β-lactamase (MBL) production. The genotype of the SMA-positive strains was confirmed to be bla_{IMP}, using an immunochromatographic assay kit (Mizuho Medy Co., Ltd; Saga, Japan). We classified MDRP and CRPA according to the presence of bla_{IMP} and compared the distributions of the MIC values.

Overall, 21 MDRP strains and 8 CRPA strains were included in the study (*Table 1*). Among these, 81%

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Antimicrobial resistance, blaIMP, carbapenemase, ceftolozane/tazobactam, metallo-β-lactamase, minimum inhibitory concentration, Pseudomonas aeruginosa.

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Table 1 - *Distributions of minimum inhibitory concentration (MIC) levels for ceftolozane/tazobactam (CTLZ/TAZ) among multidrug-resistant Pseudomonas aeruginosa (MDRP) and carbapenem-resistant Pseudomonas aeruginosa (CRPA).*

	CLSI criteria	*Resistant*	*Intermediate*	*Susceptible*		
	MIC for CTLZ/TAZ (μg/mL)	>8	8	4	2	≤1
MDRP (N=21)	*bla*$_{IMP}$–positive [n=16]	16	0	0	0	0
	bla$_{IMP}$–negative [n=5]	1	2	1	0	1
CRPA (N=8)	*bla*$_{IMP}$–positive [n=2]	2	0	0	0	0
	bla$_{IMP}$–negative [n=6]	1	1	0	1	3
Total (N=29)	*bla*$_{IMP}$–positive [n=18] (%)	18 (100)	0	0	0	0
	bla$_{IMP}$–negative [n=11] (%)	2 (18.2)	3 (27.3)	1 (9.1)	1 (9.1)	4 (36.4)
	Overall (%)	20 (69.0)	3 (10.3)	1 (3.4)	1 (3.4)	4 (13.8)

(17/21) of the MDRP strains and 25% (2/8) of the CRPA strains were resistant to CTLZ/TAZ (MIC >8 μg/mL). MDRP strains were classified into 16 *bla*$_{IMP}$-positive and 5 *bla*$_{IMP}$-negative strains. All *bla*$_{IMP}$-positive MDRP strains were resistant to CTLZ/TAZ. In contrast, *bla*$_{IMP}$-negative MDRP strains demonstrated various MIC levels, including both resistant and susceptible ranges. There were two *bla*$_{IMP}$-positive and six *bla*$_{IMP}$-negative CRPA strains. Similar to MDRP, the two *bla*$_{IMP}$-positive CRPA strains showed resistance to CTLZ/TAZ. A wide variation in the MICs for CTLZ/TAZ was also observed in the six *bla*$_{IMP}$-negative CRPA strains. In total, 69.0% (20/29) of the MDRP and CRPA strains were resistant to CTLZ/TAZ. In contrast, CTLZ/TAZ retained *in vitro* susceptibility in 54.5% (6/11) of *the bla*$_{IMP}$-negative strains.

According to Japanese national surveillance (Nakayama, 2022), *P. aeruginosa* isolated from 2006 to 2015 showed carbapenem resistance in 16.0% (275/1,716 isolates) and overall positivity of MBL in 1.3% (23/1,716 isolates). Among the carbapenem-resistant *P. aeruginosa* isolates, the MBL positivity rate was 8.4% (23/275 isolates). Thus, although the isolation rates of MBL-producing *P. aeruginosa* are not significantly high in Japan, antibiogram data on potentially available agents, such as CTLZ/TAZ, against highly resistant pathogens should be developed for empiric treatment.

According to a previous study based on data from 32 hospitals in the United States, the susceptibility rate of MDRPs to CTLZ/TAZ was high at 84.0% (n=607) (Shortridge, 2017). Additionally, a Spanish nationwide survey corroborated that 84.3% of carbapenem-resistant *P. aeruginosa* isolates remained susceptible to CTLZ/TAZ (Del Barrio-Tofinõ, 2019). However, neither report mentioned CTLZ/TAZ susceptibility in MBL-positive *P. aeruginosa*. Notably, our data clearly showed that the *bla*$_{IMP}$-positive *P. aeruginosa* strains were 100% resistant to CTLZ/TAZ, whereas nearly half of the *bla*$_{IMP}$-negative MDRP or CRPA strains were susceptible to the drug. There were three *bla*$_{IMP}$-negative strains with CTLZ/TAZ MIC of 8 μg/mL, which are interpreted as intermediate to the drug. According to the literature, an increased dose (3 g every 8 hours) or an extended infusion of CTLZ/TAZ might be clinically effective against such isolates (Navarrete-Rouco, 2022).

A limitation of this study is the small number of isolates tested. Further multi-facility studies are warranted to promote the understanding of CTLZ/TAZ susceptibility among MDRP and CRPA strains in Japan because the drug is one of the key agents for treating refractory *P. aeruginosa* infection. A lack of genetic investigation of MBL should also be mentioned. As described previously, the *bla*$_{IMP}$ genotype is prevalent in Japan (Nakayama, 2022), and we examined the positivity of *bla*$_{IMP}$ alone. Different types of MBL producers may have been present in the tested isolates.

In summary, our data suggest that *bla*$_{IMP}$-positive *P. aeruginosa* strains showed complete resistance to CTLZ/TAZ. In contrast, approximately half of the non-MBL-producing MDRP or CRPA strains remained susceptible to CTLZ/TAZ. Further investigations are needed to clarify the clinical utility of CTLZ/TAZ.

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Ethical Approval

A need for informed consent from patients was waived because the data does not include identifiable information and the study was performed as a part of routine laboratory work.

Authorship Statement

All authors meet the ICMJE authorship criteria: A. Kakehi was the principal investigator; H. Hagiya conceived of the study design; K. Iio, T. Fujimori, M. Okura, H. Minabe, and Y. Yokoyama contributed to the data collection; A. Kakehi and H. Hagiya drafted the manuscript; and Fumio Otsuka and A. Higashikage supervised the study. All authors approved the final manuscript.

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