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Diminished Susceptibility to Cefoperazone/Sulbactam and Piperacillin/Tazobactam in *Enterobacteriaceae* Due to Narrow-Spectrum β-Lactamases as Well as Omp Mutation

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Abstract

Cefoperazone/sulbactam (CSL) and piperacillin/tazobactam (TZP) are commonly used in clinical practice in China because of their excellent antimicrobial activity. CSL and TZP-nonsusceptible *Enterobacteriaceae* are typically resistant to extended-spectrum cephalosporins such as ceftriaxone (CRO). However, 11 nonrepetitive *Enterobacteriaceae* strains, which were resistant to CSL and TZP yet susceptible to CRO, were collected from January to December 2020. Antibiotic susceptibility tests and whole-genome sequencing were conducted to elucidate the mechanism for this rare phenotype. Antibiotic susceptibility tests showed that all isolates were amoxicillin/clavulanic-acid resistant and sensitive to ceftazidime, cefepime, cefepime/tazobactam, cefepime/zidebactam, ceftazidime/avibactam, and ceftolozane/tazobactam. Whole-genome sequencing revealed three of seven *Klebsiella pneumoniae* strains harbored bla_{SHV-1} only, and four harbored bla_{SHV-1} and bla_{TEM-1B}. Two *Escherichia coli* strains carried bla_{TEM-1B} only, while two *Klebsiella oxytoca* isolates harbored $bla_{OXY-1-3}$ and $bla_{OXY-1-1}$, respectively. No mutation in the β-lactamase gene and promoter sequence was found. Outer membrane protein (Omp) gene detection revealed that numerous missense mutations of OmpK36 and OmpK37 were found in all strains of *K. pneumoniae*. Numerous missense mutations of OmpK36 and OmpK35 and OmpK37 deficiency were found in one *K. oxytoca* strain, and no OmpK gene was found in the other. No Omp mutations were found in *E. coli* isolates. These results indicated that narrow spectrum β-lactamases, TEM-1, SHV-1, and OXY-1, alone or in combination with Omp mutation, contributed to the resistance to CSL and TZP in CRO-susceptible *Enterobacteriaceae*.

Antibiotic susceptibility tests

Antibiotics	Breakpoints (μg/ml)	*Klebsiella pneumoniae*							*Escherichia coli*		*Klebsiella oxytoca*	
		E1	E3	E4	E7	E9	E10	E11	E6	E8	E2	E5
CRO	≤1 ≥4	≤0.5	≤0.5	≤0.5	≤0.5	1	≤0.5	1	≤0.5	≤0.5	1	1
CAZ	≤4 ≥16	1	2	1	4	4	4	4	2	4	1	1
FEP	≤2 ≥16	1	1	0.25	1	2	2	2	0.5	2	1	1
AMC	≤8 ≥32	≥128	≥128	≥128	≥128	≥128	≥128	≥128	≥128	≥128	≥128	≥128
CSL	≤16 ≥64	64	64	64	64	≥128	128	≥128	64	128	128	≥128
TZP	≤16 ≥128	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256
FPT	≤2 ≥16	1	0.5	0.06	0.125	2	1	2	0.25	1	0.125	0.25
FPZ	≤2 ≥16	0.25	0.25	0.06	0.125	0.25	0.25	1	0.125	0.25	0.125	0.125
CZA	≤8 ≥16	1	0.5	0.25	0.25	1	0.25	1	0.5	0.5	0.5	0.25
CZT	≤2 ≥8	2	1	0.5	1	2	2	2	1	1	2	2

CRO:ceftriaxone, CAZ:ceftazidime, FEP:cefepime, AMC:amoxicillin/clavulanic-acid, CSL:cefoperazone/sulbactam, TZP:piperacillin/tazobactam, FPT:cefepime/tazobactam, FPZ:cefepime/zidebactam, CZA:ceftazidime/avibactam, CZT:ceftolozane/tazobactam

Gene sequencing results

Number	Strain	ST	β-Lactamase gene	Promoter sequence mutation	Omp mutation
E1	Kpn	45	blaSHV-1、blaTEM-1B	none	OmpK36, OmpK37
E3	Kpn	45	blaSHV-1、blaTEM-1B	none	OmpK36, OmpK37
E4	Kpn	2854	blaSHV-1	none	OmpK36, OmpK37
E7	Kpn	2358	blaSHV-1、blaTEM-1B	none	OmpK36, OmpK37
E9	Kpn	2358	blaSHV-1、blaTEM-1B	none	OmpK36, OmpK37
E10	Kpn	189	blaSHV-1	none	OmpK36, OmpK37
E11	Kpn	45	blaSHV-1	none	OmpK36, OmpK37
E6	Eco	88	blaTEM-1B	none	none
E8	Eco	409	blaTEM-1B	none	none
E2	Kox	194	blaOXY-1-3	none	OmpK36 mutations, OmpK35 and OmpK37 deficiency
E5	Kox	11	blaOXY-1-1	none	no OmpK (OmpK35, OmpK36 and OmpK37) gene found

K e y w o r d s: cefoperazone/sulbactam, piperacillin/tazobactam, TEM, SHV, OXY

Introduction

Enterobacteriaceae, such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca*, are responsible for approximately 30% of healthcare-associated infections (Stewart et al. 2021). The antibiotics cefoperazone/sulbactam (CSL) and piperacillin/tazobactam (TZP) have broad activity spectra against Gram-positive, Gram-negative, and anaerobic organisms. In China, CSL and TZP are widely used in daily clinical practice (Chen et al. 2021). However, a decreasing rate of susceptibility to CSL and TZP among *Enterobacteriaceae* threatens their continued use. Enzymes such as carbapenemases, AmpC β-lactamase, and some

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extended-spectrum β-lactamases (ESBLs) (Stewart et al. 2021), are the leading cause of CSL and TZP-resistant *Enterobacteriaceae* isolates. Many of these enzymes also hydrolyze third-generation cephalosporins and most CSL and TZP-nonsusceptible *Enterobacteriaceae* are also resistant to ceftriaxone (CRO). We recently encountered *Enterobacteriaceae* isolates that were CSL and TZP-resistant (R), but CRO-susceptible (S). Here, antibiotic susceptibility tests and the whole genome-sequencing technique were applied to explore their resistance mechanisms.

Experimental

Materials and Methods

Bacterial isolates. This retrospective study was conducted from January to December 2020 in the Department of Laboratory Medicine, Yantai Yuhuangding Hospital of Shandong Province, a 3,000-bed tertiary teaching hospital located in east China. *Enterobacteriaceae* strains resistant to CSL and TZP but sensitive to CRO were collected in routine clinical practice. All isolates intentionally collected for this study were cultured in blood agar in a 35°C incubator for 16–24 hours and then stored in skim milk in a deep freezer at –80°C until use. Duplicate isolates collected from the same patient within three months were excluded. The patients' medical records were retrospectively reviewed, and information on clinical characteristics, including age, sex, and source of infection, was collected. Approval and verbal informed consent were obtained for experimentation with human subjects due to the study's retrospective nature. The study protocol, including the verbally informed consent procedure, was approved by the Yantai Yuhuangding Hospital Ethics Committee.

Identification and antibiotic susceptibility tests. All isolates were initially identified with the VITEK®2 GN card (bioMérieux, France). Then, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics, Germany) was used to confirm the identification. All procedures were performed following the manufacturer's instructions. According to the Clinical Laboratory Standards Institute (CLSI) guidelines, the minimum inhibitory concentrations (MICs) of TZP (4–128 μg/ml) and ceftazidime (CAZ, 1–64 μg/ml) were determined by VITEK®2 AST-GN09 card (bioMérieux, France). CSL resistance was tested by the disk diffusion method on Mueller-Hinton agar (105 μg, Oxoid, UK) and then confirmed by the broth microdilution method (1–128 μg/ml, Thermo Fisher Scientific, USA). The MICs of CRO (0.5–32 μg/ml), cefepime (FEP, 0.06–128 μg/ml), amoxicillin/clavulanic acid (AMC, 0.06–128 μg/ml), cefepime/tazobactam (FPT, 0.03–64 μg/ml), cefepime/zidebactam (FPZ, 0.03–64 μg/ml), ceftazidime/avibactam (CZA, 0.03–64 μg/ml) and ceftolozane/tazobactam (CZT, 0.06–128 μg/ml) were determined by the broth microdilution method (Thermo Fisher Scientific, USA), according to the CLSI. The MIC breakpoints were interpreted according to the CLSI document M100-S30 (CLSI 2020). *E. coli* ATCC 25922 was used as a control in antibiotic-susceptibility tests.

Genome sequencing. Genomic DNA was extracted using a Genomic DNA kit (Tiangen, DP305). DNA concentration was quantified using a NanoDrop™ 2000 (Thermo Scientific, USA) spectrophotometer, and verified by agarose gel electrophoresis. Fifty ng of the extracted DNA extracted was required for library preparation. Libraries were prepared using the TruePrepTM DNA Library Prep Kit V2 for Illumina (Vazyme). The sample's DNA was simultaneously fragmented and tagged with adapters in a single "transposase" enzymatic reaction. An optimized, limited-cycle polymerase chain reaction (PCR) protocol amplified tagged DNA and added sequencing indexes. Individual libraries were assessed on the QIAxcel Advanced Automatic nucleic acid analyzer and then quantitated through quantitative real-time PCR (qPCR) using KAPA SYBR® FAST qPCR kits. Finally, the library was sequenced on an Illumina HiSeq 2500 sequencing platform (Illumina Inc., USA), and 150 bp paired-end reads were generated. Raw data were filtered to remove low-quality reads, and then clean data were assembled via SPAdes v3.13. All sequencing data were uploaded to the National Center for Biotechnology Information (NCBI) database (https://submit.ncbi.nlm.nih.gov). The β-lactamase genes and outer membrane protein (Omp) genes and mutations were identified by BLAST using the ResFinder 3.0 (https://cge.cbs.dtu.dk/services/ResFinder/) via thresholds of 90% identity and minimum length coverage of 60%. The sequence type (ST) was performed using MLST 2.0 (https://cge.cbs.dtu.dk/services/MLST/). The promoter sequence of the β-lactamase gene was annotated with a Promoter 2.0 software and compared via BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

Identification and clinical characteristics. According to the inclusion criteria, 11 nonrepetitive strains were enrolled, including seven cases of *K. pneumoniae*, two cases of *E. coli*, and two cases of *K. oxytoca*. Among the 11 strains, five were isolated from sputum, three from urine, and one from bile, pus and blood, respectively. Fifty-five percent (6/11) of the isolates were obtained from an intensive care unit, 18% (2/11) from

a neurosurgery ward, and 9% (1/11) from a stomatological ward, vascular surgery ward, and hepatobiliary ward, respectively. The age of the patients ranged from 41 to 91-years-old, with an average of 67.64 ± 15.27-years-old, of which 45% (5/11) were male and 55% (6/11) were female.

Antibiotic susceptibility tests. The results of antibiotic susceptibility tests are shown in Table I. In addition to CRO, CSL, and TZP as inclusion criteria, the antibacterial activities of CAZ, FEP, and currently available β-lactam/β-lactamase inhibitors (BL/BLIs), including AMC, FPT, FPZ, CZA, and CZT were also assayed. As shown in Table I, all isolates were susceptible to CRO, CAZ, and FEP, and MIC90 were 1, 4, and 2 μg/ml, respectively. All strains were resistant to AMC, CSL, and TZP with the MIC90 ≥ 128, ≥ 128, and ≥ 256 μg/ml, respectively. Moreover, these strains showed sensitivity to FPT, FPZ, CZA, and CZT, and the MIC90 were 2, 0.25, 1, and 2 μg/ml, respectively.

Genome sequencing. The results of gene sequencing are listed in Table II. No dominant ST was found. ST45 accounted for 42.8% (n = 3) of *K. pneumoniae*, followed by ST2358, ST2854, and ST189. The sequence types of *E. coli* were ST88 and ST409. The sequence types of *K. oxytoca* were ST194 and ST11. Three of the seven *K. pneumoniae* strains harbored $bla_{\text{SHV-1}}$ while four carried $bla_{\text{SHV-1}}$ and $bla_{\text{TEM-1B}}$. All strains of *E. coli* had the $bla_{\text{TEM-1B}}$ gene. One *K. oxytoca* isolate carried $bla_{\text{OXY-1-3}}$, and the other harbored $bla_{\text{OXY-1-1}}$. No mutation in the β-lactamase gene and promoter

Table I
Antibiotic susceptibility tests by CLSI micro broth dilution method.

Antibiotics	Breakpoints (μg/ml)	*Klebsiella pneumoniae*							*Escherichia coli*		*Klebsiella oxytoca*	
		E1	E3	E4	E7	E9	E10	E11	E6	E8	E2	E5
CRO	≤1≥4	≤0.5	≤0.5	≤0.5	≤0.5	1	≤0.5	1	≤0.5	≤0.5	1	1
CAZ	≤4≥16	1	2	1	4	4	4	4	2	4	1	1
FEP	≤2≥16	1	1	0.25	1	2	2	2	0.5	2	1	1
AMC	≤8≥32	≥128	≥128	≥128	≥128	≥128	≥128	≥128	≥128	≥128	≥128	≥128
CSL	≤16≥64	64	64	64	64	≥128	128	≥128	64	128	128	≥128
TZP	≤16≥128	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256
FPT	≤2≥16	1	0.5	0.06	0.125	2	1	2	0.25	1	0.125	0.25
FPZ	≤2≥16	0.25	0.25	0.06	0.125	0.25	0.25	1	0.125	0.25	0.125	0.125
CZA	≤8≥16	1	0.5	0.25	0.25	1	0.25	1	0.5	0.5	0.5	0.25
CZT	≤2≥8	2	1	0.5	1	2	2	2	1	1	2	2

CRO – ceftriaxone, CAZ – ceftazidime, FEP – cefepime, AMC – amoxicillin/clavulanic-acid, CSL – cefoperazone/sulbactam, TZP – piperacillin/tazobactam, FPT – cefepime/tazobactam, FPZ – cefepime/zidebactam, CZA – ceftazidime/avibactam, CZT – ceftolozane/tazobactam

Table II
Gene sequencing results.

Number	Accession	Strain	ST	β-Lactamase gene	Promoter sequence mutation	Omp mutation
E1	JAKQYI000000000	*Kpn*	45	$bla_{\text{SHV-1}}$, $bla_{\text{TEM-1B}}$	none	OmpK36, OmpK37
E3	JAKOEX000000000	*Kpn*	45	$bla_{\text{SHV-1}}$, $bla_{\text{TEM-1B}}$	none	OmpK36, OmpK37
E4	JAKOEY000000000	*Kpn*	2854	$bla_{\text{SHV-1}}$	none	OmpK36, OmpK37
E7	JAKOGA000000000	*Kpn*	2358	$bla_{\text{SHV-1}}$, $bla_{\text{TEM-1B}}$	none	OmpK36, OmpK37
E9	JAKOGC000000000	*Kpn*	2358	$bla_{\text{SHV-1}}$, $bla_{\text{TEM-1B}}$	none	OmpK36, OmpK37
E10	JAKOGD000000000	*Kpn*	189	$bla_{\text{SHV-1}}$	none	OmpK36, OmpK37
E11	JAKOPO000000000	*Kpn*	45	$bla_{\text{SHV-1}}$	none	OmpK36, OmpK37
E6	JAKOEZ000000000	*Eco*	88	$bla_{\text{TEM-1B}}$	none	none
E8	JAKOGB000000000	*Eco*	409	$bla_{\text{TEM-1B}}$	none	none
E2	JAKPCC000000000	*Kox*	194	$bla_{\text{OXY-1-3}}$	none	OmpK36 mutations, OmpK35 and OmpK37 deficiency
E5	JAKPCD000000000	*Kox*	11	$bla_{\text{OXY-1-1}}$	none	no OmpK (OmpK35, OmpK36 and OmpK37) gene found

Kpn – Klebsiella pneumoniae, *Eco – Escherichia coli*, *Kox – Klebsiella oxytoca*

sequence was found. None of the isolates possessed an ESBL enzyme, AmpC β-lactamase, or carbapenemase. Numerous missense mutations of OmpK36 and OmpK37 were found in all strains of *K. pneumoniae*, while no OmpK35 mutation was found. Numerous missense mutations of OmpK36 and OmpK35 and OmpK37 genes deficiency were found in one *K. oxytoca* strain, and no OmpK gene was found in the other. No Omp (OmpC or OmpF) point mutation was found in *E. coli* isolates.

Discussion

In recent years, the global research on β-lactamases has been focused mainly on ESBLs, AmpC β-lactamase, and carbapenemase, while some narrow-spectrum β-lactamases have been ignored. It has resulted in clinical treatment failures. In this study, none of these isolates harbored an ESBL, AmpC β-lactamase, or carbapenemase. There have been few published analyses of *Enterobacteriaceae* displaying a CSL-R/TZP-R/CRO-S resistance phenotype. It is increasingly urgent to understand the CSL/TZP-resistance due to the emergence of a CSL/TZP-resistant but 3rd generation cephalosporin-susceptible *Enterobacteriaceae* phenotype, as well as the increasing reliance on CSL and TZP as empirical treatments in daily clinical practice in China.

TEM-1, SHV-1, and OXY-1 β-lactamases are narrow-spectrum β-lactamases, which belong to group 2be in the Bush-Jacoby classification scheme (Paterson and Bonomo 2005; Kashefieh et al. 2021; Rehman et al. 2021). *Enterobacteriaceae* with TEM-1, SHV-1, or OXY-1 β-lactamase are usually susceptible to CSL and TZP. However, *Enterobacteriaceae* strains resistant to CSL and TZP yet susceptible to CRO were observed in this study. It is reported that the gene mutation-induced single amino acid substitutions at Ambler positions Met^{69}, Ser^{130}, Arg^{244}, Arg^{275}, and Asp^{276} in TEM and SHV β-lactamases may result in enzymes with reduced affinity for β-lactamase inhibitors (Ramdani-Bouguessa et al. 2011; Winkler et al. 2015). Another possible mechanism is that resistance to BL/BLIs may result from gene amplification, and subsequent hyperproduction of β-lactamase (Sun et al. 2013; Noguchi et al. 2019; Zhou et al. 2019; Hubbard et al. 2020). Mutations in the promoters have been shown to be responsible for $bla_{\text{OXY-1}}$ and $bla_{\text{SHV-1}}$ amplification (Fournier et al. 1999; Han et al. 2020). Moreover, a strong promoter, such as the Pa/Pb promoter or IS26-mediated excision and repeated insertion can also lead to the TEM-1 hyperproduction (Noguchi et al. 2019; Hubbard et al. 2020). Exposure to BL/BLIs such as TZP has been shown to induce the excision and repeated insertion of $bla_{\text{TEM-1}}$, increase the $bla_{\text{TEM-1}}$ copy number and then lead to the TEM-1 hyperproduction (Schechter et al. 2018). No mutation in the β-lactamase gene and promoter sequence was found in this study. It is speculated that the possible mechanism of resistance to CSL and TZP is the gene amplification caused by gene excision and repeated insertion. The emergence of multiple β-lactamase gene copies in genome sequence and the wide application of CSL and TZP in China support this hypothesis.

Omp is necessary for drug transport across cell membranes. A deficiency of Omp has been shown to contribute to the increase in the MIC for *Enterobacteriaceae* (Aihara et al. 2021). The current research on Omp focuses mainly on carbapenem-resistant *Enterobacteriaceae* (Tian et al. 2020), and few studies on *Enterobacteriaceae* with $bla_{\text{TEM-1B}}$, $bla_{\text{SHV-1,}}$ or $bla_{\text{OXY-1}}$ appear to have examined the prevalence of the Omp deficiency. OmpK is expressed in *Klebsiella*, and OmpK35 defects are common in isolates carrying genes encoding ESBL, while defects in OmpK36 may be more critical for carbapenem resistance (Martínez-Martínez 2008). The importance of the minor porin, OmpK37, is less clear. Except for one strain with the OmpK gene deficiency, numerous missense mutations in the porin genes OmpK36 and OmpK37 were found in almost all *Klebsiella* strains in our study. It may lead to non-functional porins and be associated with CSL and TZP resistance. OmpF and OmpC constitute the main Omps in *E. coli* (Bafna et al. 2020). No Omp mutation suggests that Omp is unnecessary for resistance against CSL and TZP in *E. coli*.

Another finding was that, although this collection of *Enterobacteriaceae* was resistant to AMC, CSL, or TZP, the newer BL/BLI combinations, FPT, FPZ, CZA, and CZT, were much more active. Previous studies have confirmed that the newer BL/BLI combinations exhibit excellent antibacterial activity against *Enterobacteriaceae*, consistent with this study (Joshi et al. 2021; Kuo et al. 2021). In addition, simultaneous analysis of the MICs of FEP, FPT, and FPZ delineated tazobactam as a distinctly less active inhibitor than zidebactam against strains producing TEM-1, SHV-1, and OXY-1 β-lactamases. It may be attributed to the dual activity of zidebactam, which can protect cefepime from hydrolysis by β-lactamases, but also bind the Gram-negative PBP2 and retain an excellent antibacterial activity (Thomson et al. 2019; Morroni et al. 2021).

This report presents the resistance to CSL and TZP in clinical isolates of *Enterobacteriaceae* possessing β-lactamases that were previously thought to be adequately inhibited by sulbactam and tazobactam. The high frequency of $bla_{\text{TEM-1}}$, $bla_{\text{SHV-1}}$, and $bla_{\text{OXY-1}}$ in the CSL and TZP-resistant isolates supported the notion that $bla_{\text{TEM-1}}$, $bla_{\text{SHV-1}}$, and $bla_{\text{OXY-1}}$, alone or in combination with Omp mutations, were important contributors

to CSL and TZP resistance. However, some limitations existed in this study. We sequenced the whole genome of the collected strains, but we did not determine the activities of β-lactamases and the Omp expression. The transferability of β-lactamase genes was not confirmed. Other characteristics, such as efflux pumps or the permeation of CSL and TZP, were not evaluated. Thus, much more work is needed to clarify the resistance mechanisms of CSL/TZP-R but CRO-S *Enterobacteriaceae*.

Conclusion

In China, the CSL/TZP-R but CRO-S phenotype of *Enterobacteriaceae* is prevalent and threatens the optimal use of CSL and TZP. TEM-1, SHV-1, and OXY-1, the most common β-lactamases, alone or in combination with Omp mutations, contribute to the resistance of CSL and TZP. Continuous monitoring and investigation of CSL/TZP-R but CRO-S isolates are needed in the current era of high CSL and TZP administration.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Aihara M, Nishida R, Akimoto M, Gotoh Y, Kiyosuke M, Uchiumi T, Nishioka M, Matsushima Y, Hayashi T, Kang D. Within-host evolution of a *Klebsiella pneumoniae* clone: selected mutations associated with the alteration of outer membrane protein expression conferred multidrug resistance. J Antimicrob Chemother. 2021 Jan 19;76(2):362–369. https://doi.org/10.1093/jac/dkaa439

Bafna JA, Sans-Serramitjana E, Acosta-Gutiérrez S, Bodrenko IV, Hörömpöli D, Berscheid A, Brötz-Oesterhelt H, Winterhalter M, Ceccarelli M. Kanamycin uptake into *Escherichia coli* is facilitated by OmpF and OmpC porin channels located in the outer membrane. ACS Infect Dis. 2020 Jul 10;6(7):1855–1865. https://doi.org/10.1021/acsinfecdis.0c00102

Chen Y, Xiang Q, Liu L. Comparison of antibiotic-associated diarrhea caused by cefoperazone/sulbactam or piperacillin/tazobactam in neurosurgery patients. J Int Med Res. 2021 May;49(5): 3000605211019661. https://doi.org/10.1177/03000605211019661

CLSI. Performance standards for antimicrobial susceptibility testing. 30th ed. CLSI supplement M100. Wayne (USA): Clinical and Laboratory Standards Institute; 2020.

Fournier B, Gravel A, Hooper DC, Roy PH. Strength and regulation of the different promoters for chromosomal beta-lactamases of *Klebsiella oxytoca*. Antimicrob Agents Chemother. 1999 Apr; 43(4): 850–855. https://doi.org/10.1128/AAC.43.4.850

Han MS, Park KS, Jeon JH, Lee JK, Lee JH, Choi EH, Lee SH. SHV Hyperproduction as a mechanism for piperacillin-tazobactam resistance in extended-spectrum cephalosporin-susceptible *Klebsiella pneumoniae*. Microb Drug Resist. 2020 Apr;26(4):334–340. https://doi.org/10.1089/mdr.2019.0079

Hubbard ATM, Mason J, Roberts P, Parry CM, Corless C, van Aartsen J, Howard A, Bulgasim I, Fraser AJ, Adams ER, et al. Piperacillin/tazobactam resistance in a clinical isolate of *Escherichia coli* due to IS*26*-mediated amplification of bla_{TEM-1B}. Nat Commun. 2020 Oct 1;11(1):4915. https://doi.org/10.1038/s41467-020-18668-2

Joshi P, Shrivastava R, Bhagwat S, Patel M. Activity of β-lactam plus β-lactam-enhancer combination cefepime/zidebactam against *Klebsiella pneumoniae* harbouring defective OmpK35/36 porins and carbapenemases. Diagn Microbiol Infect Dis. 2021 Oct;101(2):115481. https://doi.org/10.1016/j.diagmicrobio.2021.115481

Kashefieh M, Hosainzadegan H, Baghbanijavid S, Ghotaslou R. The molecular epidemiology of resistance to antibiotics among *Klebsiella pneumoniae* isolates in Azerbaijan, Iran. J Trop Med. 2021 Jul 12;2021:9195184. https://doi.org/10.1155/2021/9195184

Kuo SC, Wang YC, Tan MC, Huang WC, Shiau YR, Wang HY, Lai JF, Huang IW, Lauderdale TL. *In vitro* activity of imipenem/relebactam, meropenem/vaborbactam, ceftazidime/avibactam, cefepime/zidebactam and other novel antibiotics against imipenem-non-susceptible Gram-negative bacilli from Taiwan. J Antimicrob Chemother. 2021 Jul 15;76(8):2071–2078. https://doi.org/10.1093/jac/dkab141

Martínez-Martínez L. Extended-spectrum beta-lactamases and the permeability barrier. Clin Microbiol Infect. 2008 Jan;14(Suppl 1): 82–89. https://doi.org/10.1111/j.1469-0691.2007.01860.x

Morroni G, Bressan R, Fioriti S, D'Achille G, Mingoia M, Cirioni O, Di Bella S, Piazza A, Comandatore F, Mauri C, et al. Antimicrobial activity of aztreonam in combination with old and new β-lactamase inhibitors against MBL and ESBL co-producing Gram-Negative clinical isolates: possible options for the treatment of complicated infections. Antibiotics (Basel). 2021 Nov 3;10(11):1341. https://doi.org/10.3390/antibiotics10111341

Noguchi T, Matsumura Y, Kanahashi T, Tanaka M, Tsuchido Y, Matsumura T, Nakano S, Yamamoto M, Nagao M, Ichiyama S. Role of TEM-1 β-lactamase in the predominance of ampicillin-sulbactam-nonsusceptible *Escherichia coli* in Japan. Antimicrob Agents Chemother. 2019 Jan 29;63(2):e02366-18. https://doi.org/10.1128/AAC.02366-18

Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: A clinical update. Clin Microbiol Rev. 2005 Oct;18(4):657–686. https://doi.org/10.1128/CMR.18.4.657-686.2005

Ramdani-Bouguessa N, Manageiro V, Jones-Dias D, Ferreira E, Tazir M, Caniça M. Role of SHV β-lactamase variants in resistance of clinical *Klebsiella pneumoniae* strains to β-lactams in an Algerian hospital. J Med Microbiol. 2011 Jul;60(Pt 7):983–987. https://doi.org/10.1099/jmm.0.030577-0

Rehman N, Azam S, Ali A, Khan I, Asghar M, Ali M, Waqas M, Ullah F, Sehra GE. Molecular epidemiology of antibiotic-resistant genes and potent inhibitors against TEM, CTX-M-14, CTX-M-15, and SHV-1 proteins of *Escherichia coli* in district Peshawar, Pakistan. Saudi J Biol Sci. 2021 Nov;28(11):6568–6581. https://doi.org/10.1016/j.sjbs.2021.07.028

Schechter LM, Creely DP, Garner CD, Shortridge D, Nguyen H, Chen L, Hanson BM, Sodergren E, Weinstock GM, Dunne WM Jr, et al. Extensive gene amplification as a mechanism for piperacillin-tazobactam resistance in *Escherichia coli*. mBio. 2018 Apr 24;9(2): e00583-18. https://doi.org/10.1128/mBio.00583-18

Stewart AG, Price EP, Schabacker K, Birikmen M, Harris PNA, Choong K, Subedi S, Sarovich DS. Molecular epidemiology of third-generation cephalosporin-resistant *Enterobacteriaceae* in Southeast Queensland, Australia. Antimicrob Agents Chemother. 2021 May 18;65(6):e00130-21.
https://doi.org/10.1128/AAC.00130-21

Sun J, Wang Y, Ni YX. [Hyperproduction of TEM-1 β-lactamase mediates the resistance of *Escherichia coli* to piperacillin-tazobactam and cefoperazone] (in Chinese). Chin J Infect Chemother. 2013 May;13(3):167–172.
https://doi.org/10.16718/j.1009-7708.2013.03.003

Thomson KS, AbdelGhani S, Snyder JW, Thomson GK. Activity of cefepime-zidebactam against multidrug-resistant (MDR) Gram-Negative pathogens. Antibiotics (Basel). 2019 Mar 23;8(1):32.
https://doi.org/10.3390/antibiotics8010032

Tian X, Wang Q, Perlaza-Jiménez L, Zheng X, Zhao Y, Dhanasekaran V, Fang R, Li J, Wang C, Liu H, Lithgow T, Cao J, Zhou T. First description of antimicrobial resistance in carbapenem-susceptible *Klebsiella pneumoniae* after imipenem treatment, driven by outer membrane remodeling. BMC Microbiol. 2020 Jul 20;20(1):218.
https://doi.org/10.1186/s12866-020-01898-1

Winkler ML, Papp-Wallace KM, Taracila MA, Bonomo RA. Avibactam and inhibitor-resistant SHV β-lactamases. Antimicrob Agents Chemother. 2015 Jul;59(7):3700–3709.
https://doi.org/10.1128/AAC.04405-14

Zhou K, Tao Y, Han L, Ni Y, Sun J. Piperacillin-tazobactam (TZP) resistance in *Escherichia coli* due to hyperproduction of TEM-1 β-Lactamase mediated by the promoter *Pa/Pb*. Front Microbiol. 2019 Apr 16;10:833.
https://doi.org/10.3389/fmicb.2019.00833