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NOTE

In vitro susceptibilities of clinical isolates of *Escherichia coli* and *Klebsiella* species to CSE1034 and other β-lactams

Manu Chaudhary, Shailesh Kumar and Anurag Payasi

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Infections are becoming difficult to treat with commonly used antibiotics when caused by extended spectrum β-lactamase (ESBL)- and metallo-β-lactamase (MBL)-producing organisms.[1] In recent years, the antibiotic resistance against ESBL-producing organisms has increased at an alarming rate.[2] To overcome the antibiotic resistance caused by ESBL producers, carbapenems were introduced in clinical settings. However, carbapenem resistance among the members of the *Enterobacteriaceae* family has been reported globally.[3]

In view of the increasing antibiotic-resistance problems because of ESBL and MBL, a combination of ceftriaxone with sulbactam and EDTA, altogether termed as CSE1034, was developed, which has been found to be effective against ESBL-producing organisms.[4,5] However, its efficacy against isolates harboring MBL and ESBL + MBL is yet to be explored. Therefore, *in vitro* susceptibilities of MBL- and ESBL + MBL-positive *Escherichia coli* and *Klebsiella* spp. isolates to CSE1034 and other β-lactams were investigated.

A total of 151 isolates of phase-III clinical trial including *E. coli* (96, 63.57%), *Klebsiella* spp. (*Klebsiella pneumoniae* (34, 22.51%) and *Klebsiella oxytoca* (21, 13.90%)) were obtained from 278 patients suffering from skin and skin structure infections (SSSIs), bone and joint infections (BJIs), lower respiratory infections (LRTIs), urinary tract infections (UTIs), bacterial septicemia (BS) and chronic suppurative otitis media (CSOM) enrolled in 18 centers across India. Each isolate's identity was confirmed as described earlier.[6] All isolates were screened for ESBL and MBL by the Clinical and Laboratory Standards Institute methods (CLSI)[7] and were then subjected to characterization for the types of ESBL and MBL genes.[8–10] The susceptibility testing was performed according to the CLSI method.[7]

The antibiotics included for *in vitro* study were as follows: CSE1034 (1.5 g), ceftriaxone (1 g), piperacillin plus tazobactam (4.5 g), cefoperazone plus sulbactam (2 g) and imipenem plus cilastatin (0.5 g).

Out of the 96 *E. coli* received, 75 were from UTIs, 10 from BS, 7 from LRTIs, 3 from BJIs and 1 from CSOMs. Out of the 34 *K. pneumoniae*, 9 were from CSOMs, 8 from SSSIs, 7 from LRTIs, 4 from UTIs, 3 from BS and 3 from BJIs; of the 21 *K. oxytoca*, 7 were from CSOMs, 6 from UTIs, 4 from SSSIs, 3 from LRTIs and 1 from BS. The screening results confirmed that 50 isolates were ESBL producers, 15 were MBL producers and 86 had coproduced both ESBL and MBL. In *E. coli,* TEM-1, TEM-2 and TEM-50 were observed in 27, 17 and 7 isolates, respectively, whereas SHV-1 and SHV-10 were found in 58 and 10 isolates, respectively. NDM-1, VIM-1 and IMP-1 were found in 28, 17 and 16 isolates, respectively. Similarly, in *Klebsiella* spp., TEM-1, TEM-2 and TEM-4 were observed in 16, 18 and 2 isolates, respectively, whereas SHV-1 and SHV-2 were found in 35 and 2 isolates, respectively. NDM-1, VIM-1 and IMP-1 were found in 7, 28 and 26 isolates, respectively.

In vitro antibacterial susceptibility data are presented in Table 1. CSE1034 was the most active antibacterial agent with the majority of ESBL-producing *E. coli* displaying 93.02% susceptibility, whereas MBL- and ESBL + MBL-producing isolates exhibiting 100% susceptibility. Imipenem plus cilastatin appeared to be the second most active agent with 67.44% susceptibility, followed by piperacillin plus tazobactam (58.14%), cefoperazone plus sulbactam (55.81%) and ceftriaxone (51.16%). However, *E. coli* harboring ESBL + MBL exhibited 86.36%, 95.45%, 90.90% and 100% resistance to imipenem plus cilastatin, piperacillin plus tazobactam, cefoperazone plus sulbactam and ceftriaxone, respectively. *Klebsiella* spp. (*K. oxytoca* and *K. pneumoniae*) appeared to be highly susceptible to CSE1034 (100% to each of ESBL- and MBL-producing isolates, and 93–96% to ESBL + MBL-producing isolates). ESBL-producing *Klebsiella* spp. were equally susceptible to imipenem plus cilastatin and piperacillin plus tazobactam (66–75%), and susceptibilities of cefoperazone plus sulbactam and ceftriaxone varied from 33 to 50% and 25 to 33%, respectively. However, ESBL + MBL-producing *Klebsiella* spp. showed 100% resistance to ceftriaxone followed by 96–100% to each of the piperacillin

Department of Cell Culture and Molecular Biology, Venus Medicine Research Centre, Baddi, India
Correspondence: Dr A Payasi, Department of Cell Culture and Molecular Biology, Venus Medicine Research Centre, Hill Top Industrial Estate, Bhatoli Kalan, Baddi 173205, India.
E-mail: ccmb@venusremedies.com
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Table 1 *In vitro* activity of CSE1034 and other comparator antibiotics

		Susceptibility of clinical isolates					
		ESBL		*MBL*		*ESBL + MBL*	
Organism (number of isolates)	*antibiotics*	*% of isolate susceptibility*	*% of isolate resistant*	*% of isolate susceptibility*	*% of isolate resistant*	*% of isolate susceptibility*	*% of isolate resistant*
Escherichia coli (96)	Imipenem + cilastatin	67.44	32.55	—	100	13.64	86.36
	Piperacillin + tazobactam	58.14	41.86	—	100	4.55	95.45
	Cefoperazone + sulbactam	55.81	44.19	—	100	9.09	90.90
	CSE1034	93.02	6.98	100	—	100	—
	Ceftriaxone	51.16	48.84	—	100	—	100
Klebsiella oxytoca (21)	Imipenem + cilastatin	66.66	33.33	—	100	12.5	87.5
	Piperacillin + tazobactam	66.66	33.33	—	100	—	100
	Cefoperazone + sulbactam	33.33	66.66	—	100	—	100
	CSE1034	100	—	100	—	93.75	6.25
	Ceftriaxone	33.33	66.66	—	100	—	100
Klebsiella pneumoniae (34)	Imipenem + cilastatin	75	25	—	100	15.38	84.61
	Piperacillin + tazobactam	75	25	—	100	3.84	96.15
	Cefoperazone + sulbactam	50	50	—	100	3.84	96.15
	CSE1034	100	—	100	—	96.15	3.84
	Ceftriaxone	25	75	—	100	—	100

Abbreviations: ESBL, extended spectrum β-lactamase; MBL, metallo-β-lactamase.
Among the 96 *E. coli*, ESBL-, MBL- and ESBL + MBL-producing *E. coli* were 43, 9 and 44, respectively. Similarly, in *K. oxytoca*, ESBL-, MBL- and ESBL + MBL-producing *K. oxytoca* were 3, 2 and 16, respectively, and for *K. pneumoniae*, ESBL-, MBL- and ESBL + MBL-producing *K. pneumoniae* were 4, 4 and 26, respectively.

Table 2 MIC distributions for isolates obtained from various clinical specimens

			Number of isolates and MIC ($\mu g\,ml^{-1}$):							
Organisms (number of isolates)	*Antibiotics*	*Characteristic of isolates*	*2*	*4*	*8*	*16*	*32*	*64*	*128*	*>128*
Escherichia coli (96)	Imipenem + cilastatin	MBL						2	5	2
		ESBL			6	9	14	5	9	
		ESBL + MBL			1	2	3	10	21	7
	Piperacillin + tazobactam	MBL						1	3	5
		ESBL			2	11	12	6	4	8
		ESBL + MBL				1	1	12	13	17
	Cefoperazone + sulbactam	MBL							4	5
		ESBL			1	11	12	3	6	10
		ESBL + MBL				1	3	8	20	12
	CSE1034	MBL			2	3	4			
		ESBL		4	10	6	20	3		
		ESBL + MBL		2	16	4	22			
	Ceftriaxone	MBL							1	8
		ESBL				6	16	8	5	8
		ESBL + MBL							8	36
Klebsiella oxytoca (21)	Imipenem + cilastatin	MBL						1	1	
		ESBL				2	1	1		
		ESBL + MBL				1	1	6	4	4
	Piperacillin + tazobactam	MBL							1	1
		ESBL					2		1	
		ESBL + MBL						2	3	11
	Cefoperazone + sulbactam	MBL								2
		ESBL					1		1	1
		ESBL + MBL							2	14
	CSE1034	MBL				1	1			
		ESBL			1	2				
		ESBL + MBL			1	6	8	1		
	Ceftriaxone	MBL								2
		ESBL				1	1		1	
		ESBL + MBL							8	8
Klebsiella pneumoniae (34)	Imipenem + cilastatin	MBL						3	1	
		ESBL				2	1	1		
		ESBL + MBL				3	1	10	4	8
	Piperacillin + tazobactam	MBL						1	2	1
		ESBL					3	1		
		ESBL + MBL					1	6	10	9
	Cefoperazone + sulbactam	MBL							2	2
		ESBL				1	1		2	
		ESBL + MBL					1	4	8	13
	CSE1034	MBL				2	2			
		ESBL			1	2	1			
		ESBL + MBL			3	12	10	1		
	Ceftriaxone	MBL								4
		ESBL					1	1	1	1
		ESBL + MBL						2	4	20

Abbreviations: ESBL, extended spectrum β-lactamase; MBL, metallo-β-lactamase.

plus tazobactam and cefoperazone plus sulbactam, and 84–87% to imipenem plus cilastatin. Interestingly, the most important observation of this study was that all MBL-producing isolates (*E. coli* and *Klebsiella* spp.) were almost 100% resistant to all the comparator antibiotics.

With regard to MIC distributions (Table 2), CSE1034 MICs fell within 4–32 μg ml^{-1} against 97% of *E. coli* and 96% of *Klebsiella* spp. The MIC distributions of ceftriaxone, imipenem plus cilastatin, piperacillin plus tazobactam and cefoperazone plus sulbactam for *E. coli* isolates varied between 8 and >128. Imipenem plus cilastatin and ceftriaxone MICs varied between 16 and >128, and piperacillin plus tazobactam and cefoperazone plus sulbactam MICs were 32 to >128 for *Klebsiella* spp.

CSE1034 showed intermediate-to-resistant response to TEM-50-positive isolates. However, it appeared to be highly susceptible to NDM-1-, VIM-1- and IMP-1-positive isolates. Imipenem plus cilastatin, piperacillin plus tazobactam, cefoperazone plus sulbactam and ceftriaxone that were resistant to NDM-1-, VIM-1-, IMP-1- and TEM-50-positive isolates, however, were found to be susceptible to those isolates positive with TEM-1, TEM-2 and SHV-1. The enhanced susceptibility of CSE1034 against *E. coli* and *Klebsiella* spp. is likely to be associated with synergistic activity of ceftriaxone plus sulbactam plus EDTA.[11,12] The CSE1034 enhanced the susceptibility by altering the outer-membrane permeability, which in turn increased penetration of drug inside the bacterial cells. Furthermore, EDTA chelates the divalent ions required for the activity of MBL, thus enhancing the susceptibility of CSE1034 toward MBL-producing organisms. Our previous studies also demonstrated the enhanced *in vitro* efficacy of CSE1034.[5,6,11,13] Studies in animal models also demonstrated promising *in vivo* efficacy of CSE1034.[14,15]

The results obtained in the present study confirmed the broad-spectrum activity of CSE1034 against the MBL- and ESBL + MBL-producing organisms. Hence, CSE1034 can be considered as a drug of choice for the treatment of infections caused by these organisms.

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