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Frequency of Spontaneous Mutations Conferring Reduced Susceptibility to SPR859 (Tebipenem) Among Susceptible and Extended-Spectrum Beta-Lactamase Producing *Escherichia coli* and *Klebsiella pneumoniae*

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ABSTRACT

• **Background:** Resistance among Gram-negative uropathogenic bacteria has increased in recent years. SPR859 (tebipenem) has potent activity against *Escherichia coli* (Ec) and *Klebsiella pneumoniae* (Kp) producing extended-spectrum β-lactamases (ESBLs), and SPR994 (tebipenem pivoxil, the orally active prodrug of SPR859) is being developed as an oral therapeutic for complicated urinary tract infections (cUTI). Bacterial cells are able to acquire resistance to antibiotics via multiple routes, including by spontaneous chromosomal mutation. This study explored the frequency of spontaneous mutations (FOR) conferring reduced susceptibility to SPR859 in susceptible and ESBL producing isolates of Ec and Kp.

• **Methods:** The FOR of SPR859 and meropenem (MEM) was assessed in 5 Ec isolates, including 2 uropathogenic strains and 2 ESBL producers, and 4 Kp isolates, including 1 ESBL producer. The SPR859 MIC vs. these strains was 0.015 – 0.25 mg/L. Cultures were grown overnight, plated on cation-adjusted Mueller Hinton agar (MHAII) with SPR859 or MEM at 0.03 – 2 mg/L (2x – 8x MIC), incubated at 35°C for 3 d. Up to 4 colonies per condition were struck on MHAII sequentially for 3 d. The susceptibility of putative mutants was tested using the CLSI broth microdilution assay (MIC); isolates with a ≥ 4x increase in MIC were considered mutants. The FOR was defined as mutant CFU on MHAII + compound / total CFU plated.

• **Results:** 0.12 - 0.25 mg/L of SPR859 or MEM suppressed 4/4 Ec isolates with initial MICs of 0.015 mg/L, resulting in FOR values of <4.0E-08 to <2.3E-09. Mutants were isolated from Ec ATCC 25922 on 0.06 mg/L of both SPR859 and MEM at a frequency of 2.6E-06 with resulting MICs of 0.25 mg/L against both SPR859 and MEM; no mutants were isolated from 3/4 strains on 0.06 mg/L of either compound. Ec CDC AR0058, an ESBL+ clinical isolate with an SPR859 MIC of 0.25 mg/L, had SPR859 FORs of 1.7E-06 and 3.9E-07 and MEM FORs of 2.0E-06 and 4.3E-07 on 1 and 2 mg/L respectively; mutants had MICs of ≤ 2 mg/L to both agents. 3/4 Kp isolates had SPR859 FORs of 8.9E-07 to 1.2E-07 on 0.5 - 1 mg/L and 3.2E-07 to 9.3E-09 on 2 mg/L, while MEM FORs ranged from 8.3E-07 to <2.0E-09 on 0.5 – 2 mg/L. The 4th isolate had FORs of <1.6E-09 to both compounds at 0.5 – 2 mg/L. Confirmed Kp mutants had SPR859 and MEM MICs of 0.25 – 8 mg/mL, demonstrating cross-resistance.

• **Conclusions:** SPR859 and MEM FOR values were comparable among susceptible and ESBL+ Ec and Kp at 2.6E-06 to 9.3E-09, mutant fitness and resistance mechanisms are being evaluated. These data support continued development of SPR994, the prodrug of SPR859, as a cUTI treatment.

INTRODUCTION

Resistance among Gram-negative uropathogenic bacteria has increased in recent years. SPR859 (tebipenem) has potent activity against *Escherichia coli* (Ec) and *Klebsiella pneumoniae* (Kp) producing extended-spectrum b-lactamases (ESBLs), and SPR994 (tebipenem pivoxil, the orally active prodrug of SPR859) is being developed as an oral therapeutic for complicated urinary tract infections (cUTI). Bacterial cells are able to acquire resistance to antibiotics via multiple routes, including by spontaneous chromosomal mutation. This study explored the frequency of spontaneous mutations (FOR) conferring reduced susceptibility to SPR859 in susceptible and ESBL producing isolates of Ec and Kp.

METHODS

• The FOR of SPR859 and meropenem (MEM) was assessed in 5 Ec isolates, including 2 uropathogenic strains and 2 ESBL producers, and 4 Kp isolates, including 1 ESBL producer. The SPR859 MIC vs. these strains was 0.015 – 0.25 mg/L by CLSI method M07A10[1].

• Cultures were grown overnight, plated on cation-adjusted Mueller Hinton agar (MHAII) with SPR859 or MEM at 0.03 – 2 mg/L (2x – 8x MIC), incubated at 35°C for 3 d. Up to 4 colonies per condition were struck on MHAII sequentially for 3 d.

• The susceptibility of putative mutants was tested using a modified CLSI broth MIC assay which utilized cultures grown overnight and diluted 1:4000 (~2.0E+05 CFU/mL); isolates with a ≥ 4x increase in MIC as compared to the parent strain were considered mutants. The FOR was defined as mutant CFU on MHAII + compound / total CFU plated.

RESULTS

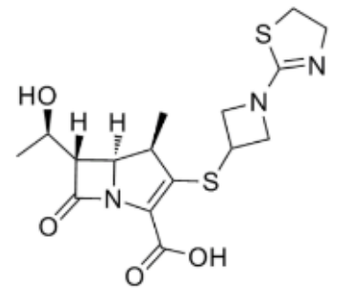
Table 1. Frequency of Spontaneous Mutations to SPR859 and MEM in *E. coli*

[compound] µg/mL	Frequency of Resistance									
	E. coli SPT-8 (ATCC 25922, antibiotic susceptible)		*E. coli* SPT-9 ATCC 35218, ESBL+		*E. coli* SPT-55, ATCC 700928, UTI model isolate		*E. coli* SPT-89, "UTI89", UTI model isolate		*E. coli* SPT-424, CDC AR Bank #58, ESBL+	
	SPR859	Meropenem	SPR859	Meropenem	SPR859	Meropenem	SPR859	Meropenem	SPR859	Meropenem
	MIC </= 0.016 - 0.03	MIC = 0.008 - 0.06	MIC </=0.016	MIC = 0.03	MIC </= 0.016 - 0.03	MIC </= 0.016 - 0.03	MIC </= 0.016 - 0.03	MIC </= 0.016 - 0.03	MIC = 0.03 - 0.25	MIC = 0.03 - 0.25
0.008	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
0.016	NS	NS	NS	NS	1.4E-06	NS	NS	NS	NS	NS
0.03	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
0.06	2.6E-06	2.6E-06	NS	NS	NS	NS	NS	NS	NS	NS
0.125	<4.0E-08	<4.0E-08	NS	<4.6E-09	<7.1E-08	<7.1E-08	NS*	NS	NS	NS
0.25	<4.0E-08	<4.0E-08	<4.6E-09	<4.6E-09	<7.1E-08	<7.1E-08	<2.3E-09	<2.3E-09	NS	NS
0.5	<4.0E-08	<4.0E-08	<4.6E-09	<4.6E-09	<7.1E-08	<7.1E-08	<2.3E-09	<2.3E-09	NS*	NS
1	<4.0E-08	<4.0E-08	<4.6E-09	<4.6E-09	<7.1E-08	<7.1E-08	<2.3E-09	<2.3E-09	1.7E-06	2.0E-06
2	<4.0E-08	<4.0E-08	<4.6E-09	<4.6E-09	<7.1E-08	<7.1E-08	<2.3E-09	<2.3E-09	3.9E-07	4.3E-07

NS = not selective (confluent growth or colonies were not confirmed as mutants)

NS* colonies were too numerous to count, individual colonies confirmed as mutants

Figure 1. Tebipenem (SPR859)



• 0.25 mg/L of SPR859 or MEM suppressed 4/4 Ec isolates with initial MICs of 0.015 mg/L, resulting in FOR values of <4.0E-08 to <2.3E-09.

• Mutants were isolated from Ec ATCC 25922 on 0.06 mg/L of both SPR859 and MEM at a frequency of 2.6E-06 with resulting MICs of 0.25 mg/L against both SPR859 and MEM; no mutants were isolated from 3/4 strains on 0.06 mg/L of either compound.

• Ec CDC AR0058, an ESBL+ clinical isolate with an SPR859 MIC of 0.25 mg/L, had SPR859 FORs of 1.7E-06 and 3.9E-07 and MEM FORs of 2.0E-06 and 4.3E-07 on 1 and 2 mg/L respectively; mutants had MICs of ≤ 2 mg/L to both agents.

Table 2. Frequency of Spontaneous Mutations to SPR859 and MEM in *K. pneumoniae*

[compound] µg/mL	Frequency of Resistance							
	K. pneumoniae SPT-14 (ATCC 700603, ESBL+)		*K. pneumoniae* SPT-75 (ATCC 27736, antibiotic susceptible)		*K. pneumoniae* SPT-378 (CDC AR Bank # 12)		*K. pneumoniae* SPT-382 (CDC AR Bank # 16)	
	SPR859	Meropenem	SPR859	Meropenem	SPR859	Meropenem	SPR859	Meropenem
	MIC = 0.06	MIC = 0.03	MIC = 0.03 - 0.06	MIC = 0.03	MIC = 0.06	MIC = 0.06	MIC = 0.03 - 0.06	MIC = 0.03
0.008	NS	NS	NS	NS	NS	NS	NS	NS
0.016	NS	NS	NS	NS	NS	NS	NS	NS
0.03	NS	NS	NS	NS	NS	NS	NS	NS
0.06	NS	NS	NS	NS	NS	NS	NS	NS
0.125	NS	NS	NS	NS	NS	NS	NS	NS
0.25	NS	NS	NS	NS	2.1E-06	NS	NS	8.3E-07
0.5	1.2E-07	1.2E-07	NS	NS	2.8E-07	6.9E-07	7.0E-07	4.5E-07
1	1.1E-07	1.1E-07	<1.6E-09	<1.6E-09	1.5E-07	2.1E-07	8.9E-07	<2.0E-09
2	1.3E-07	1.3E-07	<1.6E-09	<1.6E-09	9.3E-09	3.7E-09	3.2E-07	<2.0E-09

NS = not selective (confluent growth or colonies were not confirmed as mutants)

• 3/4 Kp isolates had SPR859 FORs of 8.9E-07 to 1.2E-07 on 0.5 - 1 mg/L and 3.2E-07 to 9.3E-09 on 2 mg/L, while MEM FORs ranged from 8.3E-07 to <2.0E-09 on 0.5 – 2 mg/L. The 4th isolate had FORs of <1.6E-09 to both compounds at 0.5 – 2 mg/L.

• Confirmed Kp mutants had SPR859 and MEM MICs of 0.25 – 8 mg/mL, demonstrating cross-resistance.

CONCLUSIONS

• SPR859 and MEM FOR values were comparable among susceptible and ESBL+ Ec and Kp at 2.6E-06 to 9.3E-09, mutant fitness and resistance mechanisms are being evaluated.

• These data support continued development of SPR994, the prodrug of SPR859, as a cUTI treatment.

REFERENCES

[1]CLSI M07-A10: Methods for Dilution Antimicrobial Susceptibility Testing for Aerobic Bacteria