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Antimicrobial Activity of Tigecycline, a Glycylcycline, Tested Against Carbapenemase-Producing Enterobacteriaceae

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ABSTRACT

Objectives:

To evaluate the in vitro activity of tigecycline (TIG) and comparator agents tested against a well-characterized collection of carbapenemase (CPase)-producing Enterobacteriaceae (ENT) collected worldwide.

Methods:

ENT strains collected through many surveillance programs from 2000-2005 were tested for susceptibility (S) against imipenem (IMI) and meropenem (MER). Isolates with MIC ≥ 2 mg/L for IMP and MER (except indole+ Proteae and *P. mirabilis*) were screened for production of metallo-ß-lactamase (MßL) and Bush group 2f ß-lactamases (2f BL) by disk approximation (DA) tests. Isolates with positive DA tests were evaluated by PCR using generic primers for IMP, VIM and SPM when DA-positive for MßL; and for KPC, SME, IMI and NmcA when DA positive for 2f BL. CPase gene sequencing and molecular typing were additionally performed to confirm CPase production and to evaluate clonality. All CPase-producers were S tested against TIG and >25 comparators by CLSI broth microdilution methods using fresh Mueller-Hinton broth.

Results:

CPase production was characterized on 104 ENT isolates among >45,000 tested during the 2000-2005 period. The collection included *K. pneumoniae* (KPN; 52), *K. oxytoca* (KOX; 8), *Enterobacter* spp. (ESP; 24), *C. freundii* (CF; 9), *S. marcescens* (SM; 7) and *E. coli* (4) from the USA (79 strains, 11 medical centers [MC], 8 cities), Italy (2 strains, 2 MC, 2 cities), Turkey (11 strains, 2 MC, 2 cities), Greece (10 strains, 1 MC) and Spain (2 strains, 1 MC). The most frequent CPase was KPC-2/3 (73 strains), followed by VIM-1 (14), IMP-1 (11), SME-2 (5) and NMC-A (1). All 2f BLs were detected in the USA while all MßLs were detected in Europe. The majority of KPC-2/3 isolates were observed among KPN from the New York City area (43 strains; >= 9 clones). However, KPC-2/3 were also observed in CF, ESP, *E. coli* and KOX from 7 MC in 5 USA cities. The antimicrobial S of the CPase-producers are summarized in the table:

	MIC_{50} / %S			
Organism (no tested)	TIG	IMP	GEN	CIPRO
Klebsiella spp. (60)	1/100	8/22	2/58	>2/15
Enterobacter spp. (24)	0.25/100	4/54	8/43	0.5/62
Citrobacter spp. (9)	0.25/100	4/100	8/0	>2/17
Serratia spp. (7)	0.5/100	>8/0	<=2/83	<=0.25/83
E. coli (4)	0.12/100	2/100	8/0	>2/25
All Enterobacteriaceae (104)	0.5/100	8/38	4/50	>2/32

Conclusions:

CPase-producing ENT showed high rates of R to all antimicrobials tested except TIG, which was very active against this significant, contemporary collection of well characterized strains (MIC_{90}, 1 mg/L; 100% S). TIG appears to be an excellent option to polymyxins for treatment of infections caused by CPase-producing ENT.

INTRODUCTION

Enterobacteriaceae represents an important cause of infections in hospitalized patients. Although broad-spectrum cephalosporins are considered excellent choices for treating infections caused by these organisms, the emergence and dissemination of extended spectrum ß-lactamases (ESBLs) has compromised the use of these agents in certain geographic regions. As a consequence, the use of carbapenems has increased significantly in some hospitals and carbapenem-resistant Gram-negative bacilli have started to emerge.

Tigecycline is a semisynthetic glycylcycline derived from minocycline. Tigecycline has documented activity against tetracycline-resistant (tet-R) Gram-positive and Gram-negative pathogens refractory by both efflux and ribosomal protection mechanisms. In addition, tigecycline does not show cross-resistance to other antimicrobial classes. In the present study, we evaluated the in vitro activity of tigecycline and comparator agents tested against a well-characterized collection of carbapenemase-producing Enterobacteriaceae collected worldwide.

MATERIALS AND METHODS

Bacterial isolates. The SENTRY Antimicrobial Surveillance Program collected >50,000 Enterobacteriaceae isolates from medical centers located in North America, Latin America and Europe in the 2000-2005 period. The isolates were consecutively collected from bloodstream infections, skin and soft tissue infections, urinary tract infections and pneumonia in hospitalized patients according to a common protocol. Additional strains were collected from the MYSTIC Program, USA. Only isolates from documented infections were included in the study. Species identification was confirmed by standard biochemical tests and the Vitek System, where necessary.

Susceptibility testing. The Enterobacteriaceae isolates were susceptibility tested against more than 25 antimicrobials by the broth microdilution procedure as described by the CLSI using validated dry-form panels manufactured by TREK Diagnostics (Cleveland, OH, USA). Interpretations of susceptibility to all antimicrobials tested were by CLSI (2006) criteria. Tigecycline breakpoints approved by the USA Food and Drug Administration (FDA) for some Enterobacteriaceae species (Susceptible/Intermediate/Resistant at ≤ 2 / 4 / ≥ 8 mg/L) were used for comparison purposes. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853 were routinely included in the testing for quality assurance.

Screening for carbapenemases. Enterobacteriaceae isolates with reduced susceptibility to imipenem and meropenem (MIC, ≥ 2 mg/L) were tested for production of carbapenemases. Indole-positive proteae and *Proteus mirabilis* were screened only when frankly resistant (MIC, ≥ 16 mg/L) to one of these compounds since these species are inherently less susceptible to carbapenems.

- Disk approximation. Potential carbapenemase producers were screened using disk approximation techniques. MßL screens were performed using imipenem, meropenem and ceftazidime as substrates and EDTA as well as 2-mercaptopropionic acid (2-MPA) as enzyme inhibitors. Screening for serine carbapenemases was achieved by a method described by Pottumarthy et al. (2003) in which imipenem and meropenem were used as substrates and clavulanic acid as the ß-lactamase inhibitor.

- PCR. Isolates with positive disk approximation test for MßL were screened for bla_{IMP}, bla_{VIM} and bla_{SPM} using PCR primers described elsewhere. Because some strains producing serine carbapenemases may have a negative disk screening test result, isolates with elevated carbapenem MIC values and negative PCR results for MßL genes were screened for presence of IMI, KPC, NMC-A and SME genes.

Gene sequencing. PCR amplicons for the carbapenemase genes were sequenced using a Sanger-based dideoxy sequencing strategy involving the incorporation of fluorescent-dye-labeled terminators into the sequencing reaction products. Sequences obtained were compared to the available sequences via NCBI BLAST search.

Epidemiological studies. Multiple isolates from the same medical center harboring carbapenemases belonging to the same enzyme family were typed using Riboprinter™ Microbial Characterization system. Isolates with identical ribotypes were further characterized by pulsed-field gel electrophoresis (PFGE).

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RESULTS

- Only 104 carbapenemase-producing isolates were detected among 50,881 Enterobacteriaceae collected by the SENTRY Program during the six-year study period (prevalence of 0.2%).

Table 1. Distribution of carbapenamase-producing Enterbacteriaceae according to the type of carbapenemase and medical center of isolation.

Organism (no. of strains)	Carbapenemase	Medical Center number(s)	Medical center location (no. of strains)	Tigecycline MIC range (mg/L)
K. pneumoniae (53)	KPC-2/3	M2, S15	New York, NY, USA (26)	0.25-2
	KPC-2/3	M6	New York, NY, USA (6)	0.12-0.5
	KPC-2	M4	Mineola, NY, USA (6)	1-2
	KPC-2	S82	New York, NY, USA (5)	0.25-0.5
	VIM-1	S66	Athens, Greece (10)	0.12-1
K. oxytoca (7)	KPC-2	M3, S117	Little Rock, AK, USA (3)	0.25-0.5
	KPC-2/3	S15	New York, NY, USA (3)	0.12-1
	KPC-3	S30	Charlottesville, VA, USA (1)	0.5
C. freundii (9)	KPC-2/3	M2, S15	New York, NY, USA (7)	0.25-2
	KPC-2	M4	Mineola, NY, USA (1)	1
	KPC-3	M18	Wilmington, DE, USA (1)	0.12
Enterobacter cloacae (22)	KPC-2/3	M2, S15	New York, NY, USA (3)	0.12-0.5
	KPC-2	S-30	Charlottesville, VA, USA (3)	0.5
	NMC-A	S15	New York, NY, USA (1)	0.12
	IMP-1	S69	Istanbul, Turkey (10)	0.25-0.5
		S68	Ankara, Turkey (1)	1
		S66	Madrid, Spain (2)	0.25-0.5
	VIM-1	S75	Genoa, Italy (1)	0.25
		S85	Catania, Italy (1)	0.25
E. gergoviae (1)	KPC-3	M2	New York, NY, USA (1)	0.25
E. hormaechei (1)	KPC-2	S15	New York, NY, USA (1)	2
Serratia marcescens (7)	KPC-2/3	M2, S15	New York, NY, USA (2)	0.5-2
	SME-1	M4	Mineola, NY, USA (1)	0.5
		S82	New York, NY, USA (1)	0.5
		M24	Seattle, WA, USA (1)	1
		S24	Houston, TX, USA (2)	0.5-1
E. coli (4)	KPC-2/3	M2, S15	New York, NY, USA (3)	0.12-1
		M8	Cleveland, OH, USA (1)	0.12

Table 2. Clonality of carbapenemase-producing Enterobacteriaceae.

Organism	Carbapenemase (no. of isolates)	Number of molecular patterns observed
K. pneumoniae	KPC-2/3 (43)	4
	VIM-1 (10)	5
K. oxytoca	KPC-2/3 (7)	4
C. freundii	KPC-2/3 (9)	8
E. cloacae	KPC-2/3 (6)	4
	IMP-1 (11)	4
	VIM-1 (4)	4
	NMC-A (1)	1
E. gergoviae	KPC-2 (1)	1
E. hormaechei	KPC-3 (1)	1
S. marcescens	KPC-2/3 (2)	2
	SME-1 (4)	4
E. coli	KPC-2/3 (4)	4

Table 3. Antimicrobial activity of tigecycline and comparators against carbapenemase-producing Enterobacteriaceae.

Organism	MIC_{50}	MIC_{90}	% Susceptible	% Resistant
Klebsiella spp. (60)				
Tigecycline	1	2	100.0	0.0
Imipenem	8	16	21.7	46.7
Meropenem	>8	>8	20.0	61.7
Pip/tazo	>64	>64	0.0	95.8
Cefepime	>16	>16	18.8	64.6
Aztrenam	>16	>16	10.4	87.5
Ciprofloxacin	>4	>4	14.6	79.2
Gentamicin	≤2	>8	58.3	31.2
Amikacin	16	>32	53.3	13.3
Polymyxin B	≤1	≤1	93.3	6.7
Enterobacter spp. (24)				
Tigecycline	0.25	0.5	100.0	0.0
Imipenem	4	>8	54.2	17.7
Meropenem	8	>8	33.3	41.7
Pip/tazo	32	>64	19.0	42.9
Cefepime	>16	>16	9.5	85.7
Aztrenam	>16	>16	28.6	57.1
Ciprofloxacin	0.5	>4	61.9	33.3
Gentamicin	8	>8	42.9	38.1
Amikacin	2	16	95.2	4.8
Polymyxin B	≤1	≤1	95.2	4.8
All Enterobacteriaceae (104)				
Tigecycline	0.5	1	100.0	0.0
Imipenem	8	>8	37.5	37.5
Meropenem	8	>8	32.7	50.0
Pip/tazo	>64	>64	10.7	76.2
Cefepime	>16	>16	26.2	59.5
Aztrenam	>16	>16	15.5	78.6
Ciprofloxacin	4	>4	32.1	60.7
Gentamicin	4	>8	50.0	33.3
Amikacin	8	32	73.3	8.3
Polymyxin B	≤1	>4	88.1	11.9

- The most frequent carbapenemase-producing species was *K. pneumoniae* (53 strains), followed by *Enterobacter cloacae* (22) and *Citrobacter freundii* (nine strains; Table 1).

- KPC-2 or -3 harboring strains represented 70.2% (73 strains) of carbapenemase-producing Enterobacteriaceae, and 87.7% of those (64 strains) were from medical centers located in the New York City area (Table 1).

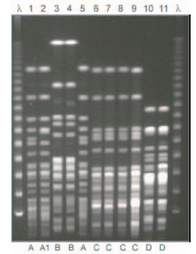
- All Bush Group 2f-producing strains were detected in the USA while all MßL-producing strains were observed in Europe (Table 1).

- MßL-producing strains were collected mainly in Athens, Greece; Ankara and Istanbul, Turkey; and Genoa and Catania, Italy; geographic areas where MßL-producing *P. aeruginosa* strains are endemic (Table 1).

- A great clonal variability was observed among carbapenemase-producing strains of the same species isolated in the same geographic area, indicating horizontal dissemination of carbapenemase genes (Table 2 and Figure 1). In addition, both intra- and inter-hospital dissemination of carbapenemase-producing strains were identified, mainly in the New York City area.

- Carbapenemase-producing Enterobacteriaceae showed high rates of resistance to most antimicrobial agents tested. The rank order of in vitro activity against these strains was: tigecycline (100.0% susceptible) > polymyxin B (88.1%) > amikacin (73.0%) > imipenem (37.5%)(Table 3).

Figure 1. PFGE profiles of KPC-2/3 producing Enterobacteriaceae isolates. Lanes λ: 48.5 Kb λ ladder; Lanes 1- 2: *K. pneumoniae* from the New York City area (M2); lanes 3-4: *K. oxytoca* from Little Rock, AK (M3); lane 5: *K. pneumoniae* from the New York City area (Mineola, M4); lanes 6-9: *K. pneumoniae* from the New York City area (M6); lanes 10-11: *C. freundii* from the New York City area (M2). The letters at the bottom of the lanes represent PFGE pattern designations.



CONCLUSIONS

- Tigecycline was most active antimicrobial tested (MIC_{50}, 0.5 mg/L and MIC_{90}, 1 mg/L) against this collection of carbapenemase-producing Enterobacteriaceae. All isolates were inhibited at the susceptible breakpoint approved by the USA-FDA for Enterobacteriaceae (≤ 2 mg/L).

- Carbapenemase-producing Enterobacteriaceae strains are still extremely rare in most regions of the world, but KPC-producing strains have become endemic and highly prevalent in the New York City area.

- MßL-producing Enterobacteriaceae have emerged in geographic areas where MßL-producing *P. aeruginosa* strains are endemic, such as Greece, Turkey, Italy, and more recently, Spain.

- Although clonal dissemination of carbapenemase-producing strains was observed in some medical centers, horizontal gene transfer seems to be a major factor in the dissemination of these resistance mechanisms.

- Carbapenemases-producing Enterobacteriaceae strains are usually resistant to many antimicrobial agents available for clinical use. Tigecycline (100.0% susceptibility) and the polymyxins (88.1% susceptibility) represent the most active antimicrobials against these troublesome organisms.