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Antimicrobial Activity of Tigecycline (TIG) Tested Against Serine Carbapenemase-Producing Enterobacteriaceae Isolated in the United States

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ABSTRACT

Background:
The activity of TIG was tested against well-characterized Bush group 2f ß-lactamase (2f BL)-producing Enterobacteriaceae (ENT) collected in USA medical centers (MC).

Methods:
ENT strains collected from 2000-2005 were susceptibility (S) tested against >30 agents by CSLI methods. Isolates with MIC ≥2 µg/ml for imipenem (IMI) and meropenem (MEM) were screened for production 2f BL by disk approximation tests and by PCR using generic primers for KPC, SME, IMI and NMC-A. 2f BL gene sequencing and molecular typing were additionally performed to confirm 2f BL production and to evaluate clonality. All 2f BL-producers were tested against TIG and >25 comparators using fresh Mueller-Hinton broth.

Results:
17,868 ENT were evaluated during the study period and 2f BL production was detected in 79 strains (0.4%). The collection included *K. pneumoniae* (KPN; 43), *K. oxytoca* (KOX; 7), *Enterobacter* spp. (ESP; 9), *C. freundii* (CF; 9), *S. marcescens* (SM; 7) and *E. coli* (4) from 11 MCs in 8 cities. KPC-2/3 was characterized in 73 (92.4%), SME-2 in 5 (all SM) and NMC-A in 1. 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 was also observed in CF, ESP, *E. coli* and KOX from 7 MC in 5 USA cities. The antimicrobial S results of the 2f BL-producers are summarized in the table:

	MIC_{50} / %S				
Organism (no tested)	TIG	IMP	MEM	Gentamicin	Ciprofloxacin
Klebsiella spp. (50)	0.5/100	8/22	>8/18	4/52	>2/14
C. freundii (9)	0.25/100	4/100	4/100	>8/0	>2/22
Enterobacter spp. (9)	0.5/100	8/33	>8/33	>8/22	>2/22
S. marcescens (7)	0.5/100	>8/0	>8/14	≤2/86	≤0.25/71
E. coli (4)	0.12/100	2/100	2/100	8/25	>2/25
All ENT (79)	0.5/100	8/34	>8/33	8/44	>2/22

Conclusions:
2f BL-producing ENT showed high R rates to all antimicrobials tested except TIG, which was very active against this emerging, contemporary collection of well characterized strains (MIC_{90}, 1 µg/ml; 100% S). TIG appears to be an excellent alternative to polymyxins for treatment of infections caused by 2f BL-producing ENT.

INTRODUCTION

Until recently, carbapenem resistance was very uncommon among Enterobacteriaceae isolates with occasional reports of strains with carbapenem resistance due to hyperproduction of AmpC cephalosporinase, which possesses relatively weak carbapenemase activity, combined with reduced porin expression or over-expressed efflux pumps. Thus, broad-spectrum cephalosporins were considered excellent choices for treating infections caused by these organisms.

The emergence and dissemination of extended spectrum ß-lactamases (ESBLs) has compromised, however, the use of these cephem agents in certain geographic regions. As a consequence, the therapeutic use of carbapenems has increased significantly in some hospitals and carbapenem-resistant Gram-negative bacilli have begun to emerge. More recently, serine carbapenemases, mainly the KPC enzymes, have been increasingly reported in several pathogens, including *Escherichia coli*, *Klebsiella* species, *Enterobacter* species, and *Salmonella enterica*. KPC-type genes reside on transmissible plasmids, which facilitates dissemination of this important mechanism of ß-lactam resistance.

Tigecycline was recently approved by the United States (US) Food and Drug Administration (FDA) for treatment of skin and soft tissue and intra-abdominal infections. 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 this study, we evaluated the activity of tigecycline against a well-characterized collection of Bush group 2f ß-lactamase-producing Enterobacteriaceae strains collected in US medical centers.

MATERIALS AND METHODS

Bacterial isolates. A total of 17,868 Enterobacteriaceae isolates were collected from medical centers located in North America during 2000-2005. Among those, the production of serine carbapenemases (Bush group 2f ß-lactamases) was detected in 79 (0.4%) strains, which were further evaluated in the present study. The isolates were consecutively obtained from bloodstream infections, skin and soft tissue infections, urinary tract infections and pneumonia in hospitalized patients. Only isolates from documented infections (non-duplicate) were included in the study. Species identification was confirmed by standard biochemical tests and the Vitek System (bioMerieux, Hazelwood, MO).

Susceptibility testing. All isolates were susceptibility tested using the broth microdilution method as specified by the CLSI (formerly NCCLS). Cation-adjusted Mueller-Hinton broth was used in validated panels manufactured by TREK Diagnostics (Cleveland, OH). Categorical interpretations for comparator antimicrobials were those of M100-S16 (2006); breakpoints for Enterobacteriaceae when testing tigecycline were those of the US-FDA (≤ 2 / ≥ 8 µg/ml for susceptible/resistant). Quality control (QC) was performed using *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853; all QC results were within ranges as specified in M100-S16.

Phenotypic detection of carbapenemase-producing strains. Any isolate with reduced susceptibility to imipenem and meropenem (MIC, ≥2 µg/ml) was further tested for production of inactivating enzymes. Because indole-positive *Proteae* and *Proteus mirabilis* are inherently less susceptible to carbapenems, only strains displaying overt resistance (MIC, ≥16 µg/ml) to both compounds were tested further.

Screening for serine carbapenemases was performed using a disk approximation (DA) technique described by Pottumarthy et al. (2003) in which imipenem and meropenem were used as substrates and clavulanic acid as the ß-lactamase inhibitor.

Genotypic detection of serine carbapenemases. Because some strains producing serine carbapenemases may have a negative DA screening test result, isolates with elevated carbapenem MIC values were also screened for presence of IMI, KPC, NMC-A and SME genes using corresponding published primer sets. 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 harbouring carbapenemases belonging to the same enzyme family were typed using the Riboprinter™ Microbial Characterization system (DuPont Qualicon; Wilmington, DE). Isolates with identical ribotypes were further characterized by pulsed-field gel electrophoresis (PFGE).

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RESULTS

- Among 17,868 Enterobacteriaceae collected during the six-year study period (2000-2005), only 79 serine carbapenemase-producing strains (0.4%) were detected.

Table 1. Antimicrobial activity of tigecycline and comparators tested against serine carbapenemase-producing Enterobacteriaceae.

	MIC (µg/ml)			
Antimicrobial (no. tested)	MIC_{50}	MIC_{90}	% Susceptible	% Resistant
All Enterobacteriaceae (79)				
Tigecycline	0.5	2	100.0	0.0
Imipenem	8	>8	34.2	44.3
Meropenem	>8	>8	32.9	55.7
Piperacillin/tazobactam	>64	>64	7.6	84.8
Ceftazidime	>16	>16	17.7	78.5
Aztreonam	>16	>16	5.1	92.4
Ciprofloxacin	>4	>4	21.5	70.9
Gentamicin	8	>8	44.3	40.5
Klebsiella spp. (50)				
Tigecycline	0.5	2	100.0	0.0
Imipenem	8	>8	22.0	48.0
Meropenem	>8	>8	18.0	66.0
Piperacillin/tazobactam	>64	>64	0.0	94.0
Ceftazidime	>16	>16	10.0	88.0
Aztreonam	>16	>16	2.0	96.0
Ciprofloxacin	>4	>4	14.0	80.0
Gentamicin	4	>8	52.0	36.0
C. freundii (9)				
Tigecycline	0.25	-	100.0	0.0
Imipenem	4	-	100.0	0.0
Meropenem	4	-	100.0	0.0
Piperacillin/tazobactam	>64	-	0.0	88.9
Ceftazidime	>16	-	0.0	88.9
Aztreonam	>16	-	0.0	100.0
Ciprofloxacin	>4	-	22.2	66.7
Gentamicin	>8	-	0.0	55.6
Enterobacter spp. (9)				
Tigecycline	0.5	-	100.0	0.0
Imipenem	8	-	33.3	44.4
Meropenem	>8	-	33.3	66.7
Piperacillin/tazobactam	>64	-	11.1	88.9
Ceftazidime	>16	-	22.2	77.8
Aztreonam	>16	-	11.1	88.9
Ciprofloxacin	4	-	22.2	77.8
Gentamicin	>8	-	22.2	77.8
S. marcescens (7)				
Tigecycline	0.5	-	100.0	0.0
Imipenem	>8	-	0.0	100.0
Meropenem	>8	-	14.3	71.4
Piperacillin/tazobactam	2	-	71.4	28.6
Ceftazidime	≤1	-	71.4	14.3
Aztreonam	>16	-	28.6	57.1
Ciprofloxacin	≤0.25	-	71.4	0.0
Gentamicin	≤2	-	85.7	0.0
E. coli (4)				
Tigecycline	0.12	-	100.0	0.0
Imipenem	2	-	100.0	0.0
Meropenem	2	-	100.0	0.0
Piperacillin/tazobactam	64	-	0.0	50.0
Ceftazidime	4	-	50.0	50.0
Aztreonam	>16	-	0.0	100.0
Ciprofloxacin	>4	-	25.0	75.0
Gentamicin	8	-	25.0	50.0

- Rates of resistance to most antimicrobial agents tested were very elevated among serine carbapenemase-producing Enterobacteriaceae, but tigecycline was highly active against this group of multidrug-resistant strains (MIC_{50}, 0.5 µg/ml; 100.0% susceptible; Table 1).
- Tigecycline showed excellent in vitro activity against KPC-producing *Klebsiella* spp. (MIC_{50}, 0.5 µg/ml; MIC_{90}, 2 µg/ml; 100.0% susceptible). The second most active compound tested against *Klebsiella* spp. was gentamicin (MIC_{50}, 4 µg/ml; 52.0% susceptible; Table 1).
- *Enterobacter* spp. showed very low rates of susceptibility to all compounds tested except tigecycline (100.0% susceptible). Susceptibility rates to other compounds varied from 11.1 to 33.3% (Table 1).
- In spite of serine carbapenemase production, approximately one-third of the strains showed imipenem and/or meropenem MIC results within the CLSI susceptible range (Table 1).

Table 2. Distribution of serine carbapenemase types listed by bacterial species and medical centers.

Carbapenamase	Organisms (no. of strains)	No. of molecular patterns	Medical center location (no. of medical centers/ no. of strains)
KPC-2 or -3	*Citrobacter freundii* (9)	8	New York City area (2/8) Wilmington, DE (1/1)
	Enterobacter cloacae (6)	4	Charlottesville, VA (1/3) New York City area (1/3)
	Enterobacter gergoviae (1)	1	New York City area (1/1)
	Enterobacter hormaechei (1)	1	New York City area (1/1)
	Escherichia coli (4)	4	Cleveland, OH (1/1) New York City area (1/3)
	Klebsiella oxytoca (7)	4	Charlottesville, VA (1/1) Little Rock, AK (1/3) New York City area (1/3)
	Klebsiella pneumoniae (43)	4	New York City area (4/43)
	Serratia marcescens (2)	2	New York City area (1/2)
NMC-A	*Enterobacter cloacae* (1)	1	New York City area (1/1)
SME-2	*Serratia marcescens* (5)	5	Akron, OH (1/1) Houston, TX (1/2) New York City area (1/1) Seattle, WA (1/1)

- The serine carbapenemase-producing isolates were observed in 11 medical centers located in 8 US cities (Table 2).
- The most frequent serine carbapenemase-producing species was *K. pneumoniae* (43 strains), followed by *Citrobacter freundii* (9 strains) and *Enterobacter aerogenes*, *K. oxytoca* and *S. marcescens* with 7 strains each (Table 2).
- KPC-2 or -3 harbouring strains represented 92.4% (73 strains) of serine carbapenemase-producing Enterobacteriaceae, and 87.7% of those (64 strains) were from medical centers located in the New York City area (Table 2).
- Serine carbapenemase-producing strains of the same species isolated in the same geographic area showed significant clonal variability, indicating horizontal dissemination of carbapenemase genes (Table 2). Furthermore, clonal dissemination of serine carbapenemase-producing strains was identified in the New York City area; however, these enzymes have been observed in other remote locations.

CONCLUSIONS

- All serine carbapenemase-producing Enterobacteriaceae isolates were inhibited at the tigecycline susceptible breakpoint approved by the US-FDA (≤2 µg/ml). This compound was the most active antimicrobial tested against this collection of multidrug-resistant strains (MIC_{50}, 0.5 µg/ml and MIC_{90}, 2 µg/ml).
- KPC-producing Enterobacteriaceae strains have become endemic and highly prevalent in the New York City area.
- The widespread dissemination of serine carbapenemase-producing Enterobacteriaceae has profound implications regarding the continued clinical utility of the carbapenems.
- Serine carbapenemase-producing Enterobacteriaceae strains were generally resistant to the vast majority of antimicrobial agents available for clinical use. Tigecycline (100.0% susceptibility) represents an active alternative antimicrobial against these multidrug-resistant organisms.