

Antimicrobial Activities of Tigecycline and Other Broad-Spectrum Antimicrobials Tested against Serine Carbapenemase- and Metallo- β -Lactamase-Producing *Enterobacteriaceae*: Report from the SENTRY Antimicrobial Surveillance Program[∇]

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Received 23 August 2007/Returned for modification 23 October 2007/Accepted 23 November 2007

A total of 104 carbapenemase (serine- and metallo- β -lactamase [M β L])-producing strains of the *Enterobacteriaceae* family collected from 2000 to 2005 in medical centers distributed worldwide were tested against tigecycline and 25 comparators by reference broth microdilution methods. The most frequent carbapenemase was KPC-2 or -3 (73 strains), followed by VIM-1 (14), IMP-1 (11), SME-2 (5), and NMC-A (1). All serine carbapenemases were detected in the United States, while M β L-producing strains were isolated in Europe. Carbapenemase-producing *Enterobacteriaceae* showed high rates of resistance to most antimicrobial agents tested. The rank order of in vitro activity against these strains was as follows: tigecycline (100.0% susceptible) > polymyxin B (88.1%) > amikacin (73.0%) > imipenem (37.5%). Tigecycline was very active (MIC₉₀, 1 μ g/ml) against this significant, contemporary collection of well-characterized strains and appears to be an excellent option compared to the polymyxins for treatment of infections caused by these multidrug-resistant *Enterobacteriaceae*.

The emergence and dissemination of extended-spectrum β -lactamases has compromised the use of broad-spectrum cephalosporins for empirical treatment of hospitalized patients' infections caused by various member of the *Enterobacteriaceae*. As a consequence, the therapeutic use of carbapenems has increased significantly in some hospitals, and carbapenem-resistant gram-negative bacilli have begun to emerge (7).

Resistance to carbapenems in *Enterobacteriaceae* can be caused by overproduction of Amp-C β -lactamases associated with loss of outer membrane porins and/or overexpression of efflux pumps (12) or by production of β -lactamases with significant hydrolysis activity against carbapenem compounds. These carbapenemases can be divided into the metallo- β -lactamases (M β L; Ambler class B) and serine carbapenemases (class A or Bush class 2f) according to the functional requirements and the structure of their active site (15, 17). The genes encoding most of these carbapenemases reside on plasmids or transposons carrying additional genes encoding resistance to other classes of antimicrobial agents (13). These transferable structures can readily be acquired by gram-negative pathogens, facilitating the dissemination of these potent resistance mechanisms and, in many cases, conferring on the isolate a multidrug resistance profile (18), significantly reducing the treat-

ment options for infections caused by carbapenemase-producing isolates.

Tigecycline is a semisynthetic glycylcycline derived from minocycline that has documented activity against tetracycline-resistant gram-negative pathogens that are refractory as a result of both efflux and ribosomal protection mechanisms (10). In addition, organisms that are resistant to other antimicrobial classes do not exhibit cross-resistance to tigecycline, supporting the potential therapeutic use of this antimicrobial agent for the treatment of infections caused by carbapenemase-producing *Enterobacteriaceae* isolates (9).

In the present study, we tested the in vitro activity of tigecycline and comparator agents against a well-characterized collection of carbapenemase-producing *Enterobacteriaceae* collected worldwide.

MATERIALS AND METHODS

Bacterial isolates. The SENTRY Antimicrobial Surveillance Program collected *Enterobacteriaceae* isolates from medical centers located in North America, Latin America, and Europe for the interval from 2000 to 2005. 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 (United States). Only isolates from documented infections were included in these studies. Species identification was confirmed by standard biochemical tests and the Vitek system (bioMérieux, Hazelwood, MO) when necessary.

Antimicrobial susceptibility testing. All isolates were susceptibility tested by the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) (5). Fresh cation-adjusted Mueller-Hinton broth was used in validated panels manufactured by TREK Diagnostics (Cleveland, OH). Categorical interpretations for comparator antimicrobials were those found in M100-S17 (4); breakpoints for *Enterobacteriaceae* when testing

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[∇] Published ahead of print on 10 December 2007.

TABLE 1. Distribution of carbapenemase-producing *Enterobacteriaceae* isolates according to carbapenemase type and medical center location

Organism (no. of strains)	Carbapenemase	Medical center		Tigecycline MIC range ($\mu\text{g/ml}$)
		No.	Location (no. of strains)	
<i>K. pneumoniae</i> (53)	KPC-2/3	4	New York, NY (37)	0.25–4
	KPC-2	1	Mineola, NY (6)	1–2
	VIM-1	1	Athens, Greece (10)	0.12–1
<i>K. oxytoca</i> (7)	KPC-2	2	Little Rock, AK (3)	0.25–0.5
	KPC-2/3	1	New York, NY (3)	0.12–1
	KPC-3	1	Charlottesville, VA (1)	0.5
<i>C. freundii</i> (9)	KPC-2/3	2	New York, NY (7)	0.25–2
	KPC-2	1	Mineola, NY (1)	1
	KPC-3	1	Wilmington, DE (1)	0.12
<i>E. cloacae</i> (22)	KPC-2/3	2	New York, NY (3)	0.12–0.5
	KPC-2	1	Charlottesville, VA (3)	0.5
	NMC-A	1	New York, NY (1)	0.12
	IMP-1	1	Istanbul, Turkey (10)	0.25–0.5
		1	Ankara, Turkey (1)	1
		1	Madrid, Spain (2)	0.25–0.5
	VIM-1	1	Genoa, Italy (1)	0.25
		1	Catania, Italy (1)	0.25
<i>E. gergoviae</i> (1)	KPC-3	1	New York, NY (1)	0.25
<i>E. hormaechei</i> (1)	KPC-2	1	New York, NY (1)	2
<i>S. marcescens</i> (7)	KPC-2/3	2	New York, NY (2)	0.5–2
	SME-1	1	Mineola, NY (1)	0.5
		1	New York, NY (1)	0.5
		1	Seattle, WA (1)	1
		1	Houston, TX (2)	0.5–1
<i>E. coli</i> (4)	KPC-2/3	2	New York, NY (3)	0.12–1
		1	Cleveland, OH (1)	0.12

tigecycline were those of the U.S. Food and Drug Administration (≤ 2 and ≥ 8 $\mu\text{g/ml}$ for susceptible and resistant, respectively). Quality control was performed with *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853; all quality control results were within specified ranges as published in M100-S17 (4).

Phenotypic detection of carbapenemase-producing strains. *Enterobacteriaceae* isolates with reduced susceptibility to imipenem or meropenem (MIC, ≥ 2 $\mu\text{g/ml}$) were tested for production of carbapenemases. Indole-positive members of the tribe *Proteae* and strains of *Proteus mirabilis* were screened only when frankly resistant (MIC, ≥ 16 $\mu\text{g/ml}$) to one of these compounds, since these organisms are inherently less susceptible to carbapenems.

Potential carbapenemase producers were screened by using disk approximation techniques. M β L screening was performed using imipenem, meropenem, and ceftazidime as substrates and using EDTA and 2-mercaptopyruvic acid as enzyme inhibitors (1). Screening for serine carbapenemases was carried out by a method described by Pottumarthy et al. (14), in which imipenem and meropenem were used as substrates and clavulanic acid as the β -lactamase inhibitor.

Genotypic detection of carbapenemases. Isolates with positive disk approximation tests for M β L were screened for *bla*_{IMP}, *bla*_{VIM}, and *bla*_{SPM} by PCR. Because some strains producing serine carbapenemases may have a negative disk screening result, isolates with elevated carbapenem MICs and negative PCR for M β L genes were screened for the presence of IMI, KPC, NMC-A, and SME genes. PCR amplicons were sequenced, and the DNA sequences obtained were compared to the available sequences via National Center for Biotechnology Information BLAST search.

Epidemiological studies. Multiple isolates from the same medical center harboring the same carbapenemase-encoding gene were typed with a Riboprinter microbial characterization system (DuPont Qualicon, Wilmington, DE). Isolates with identical ribotypes were further characterized by pulsed-field gel electrophoresis (PFGE).

RESULTS

Among 50,881 *Enterobacteriaceae* collected in the 6-year period (2000 to 2005), a total of 261 isolates (0.5%) showed reduced susceptibility to imipenem or meropenem (MIC, ≥ 2 $\mu\text{g/ml}$) and were screened for production of carbapenemases. Genes encoding serine or metallo- β -lactamases were detected in 104 isolates (39.8%), showing an overall prevalence of 0.2%. The resistance mechanisms of the remaining strains are under investigation.

KPC-2 and -3 were the most commonly found carbapenemases, detected in 73 (70.2%) strains. Of note, 87.7% of those (64 strains) were recovered from medical centers in the New York City, NY, area (Table 1). KPC-producing isolates belonged to several species, including *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Citrobacter freundii*, three *Enterobacter* species, *E. coli*, and *Serratia marcescens*. VIM-1 was detected in 14 (13.5%) carbapenemase-producing strains, followed by IMP-1 in 11 (10.5%) strains. SME-1 was observed in 5 (4.8%) isolates, while NMC-A was detected in only 1 strain (1.0%). The most frequently isolated carbapenemase-producing species was *K. pneumoniae* (53 strains; 51.0%), followed by *Enterobacter cloacae* (22 strains; 21.2%) and *C. freundii* (9 strains; 8.7%). *K. oxytoca*, *S. marcescens*, *E. coli*, and other *Enterobacter* species (*Enterobacter gergov-*

iae and *Enterobacter hormaechei*) were also found among the carbapenemase-producing isolates (Table 1).

All serine carbapenemase-producing isolates were detected in the United States (10 medical centers and 8 cities), whereas MBL-producing strains were observed only in Europe (Table 1). MBL isolates were detected in Athens, Greece (10 VIM-1-producing strains); Ankara and Istanbul, Turkey (1 and 10 IMP-1 producers, respectively); Genoa and Catania, Italy (1 VIM-1-producing strain each); and Madrid, Spain (2 VIM-1-producing isolates).

Four ribotype groups were observed among KPC-producing *K. pneumoniae* isolates. KPC-2-producing strains belonged to three ribogroups, while all KPC-3-producing *K. pneumoniae* isolates belonged to a unique ribogroup. KPC-2-producing isolates from two ribogroups showed a unique PFGE type, suggesting a common ancestor (data not shown). Five of 10 VIM-1-producing *K. pneumoniae* isolates belonged to one clone (identified by ribotyping and PFGE), and two other isolates were part of another epidemic clone. The three remaining VIM-1-producing isolates from Athens were distinct from each other and from the clones observed in this geographic region (Europe).

High genetic variability was demonstrated among KPC-producing *E. cloacae* strains compared to *K. pneumoniae* strains producing the same enzyme. All KPC-producing strains showed distinct molecular typing patterns (three strains from two hospitals). Similarly, all three KPC-2-producing *E. cloacae* strains from two other hospitals were genetically distinct. In contrast, the 10 IMP-1-producing *E. cloacae* isolates from Istanbul belonged to three clones, two of them including four strains. The VIM-1-producing *E. cloacae* isolate from Ankara was distinct from isolates collected in Istanbul. Two VIM-1-producing *E. cloacae* strains from Madrid were identical to each other but different from isolates recovered in Italy (Catania and Genoa).

A considerable degree of clonal variability was observed among carbapenemase-producing strains from other species. Eight distinct molecular patterns were observed among nine KPC-producing *C. freundii* isolates from four hospitals. All *E. coli* and *S. marcescens* isolates were considered genetically unrelated by both typing methods (ribotyping and PFGE).

As expected, rates of susceptibility to most antimicrobial agents tested were very low among carbapenemase-producing isolates of *Enterobacteriaceae* (Table 2). Tigecycline was the only antimicrobial agent that inhibited 100% of these multi-drug-resistant strains (MIC₅₀, 0.5 µg/ml; 100.0% susceptible), and polymyxin B also showed good activity against carbapenemase-producing *Enterobacteriaceae* (MIC₅₀, ≤1 µg/ml; 88.1% susceptible). Amikacin was the third most active compound in vitro, with an overall 73.3% susceptible rate.

Only 7.7% of strains were susceptible to ertapenem, while imipenem and meropenem remained active against 37.5 and 32.7% of the isolates, respectively, at the current CLSI susceptibility breakpoints (5). The remaining β-lactams tested showed limited activity against these carbapenemase-producing *Enterobacteriaceae* (susceptibilities ranging from only 10.7 to 26.2% [Table 2]).

Klebsiella isolates showed lower rates of susceptibility to all compounds tested except tigecycline and polymyxin B (MIC₅₀, 1 and ≤1 µg/ml; 100.0 and 93.3% susceptible, respectively).

TABLE 2. Antimicrobial activities of tigecycline and comparators against carbapenemase-producing *Enterobacteriaceae*

Organism (no. tested) and agent	MIC ₅₀	MIC ₉₀	% Susceptible ^a	% Resistant ^a
<i>All Enterobacteriaceae</i> (104)				
Tigecycline	0.5	1	100.0	0.0
Tetracycline	4	>8	35.6	19.2
Imipenem	8	>8	37.5	37.5
Meropenem	8	>8	32.7	50.0
Ertapenem	8	32	7.7	73.1
Piperacillin-tazobactam	>64	>64	10.7	76.2
Cefepime	>16	>16	26.2	59.5
Aztreonam	>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 ^b	11.9 ^b
<i>Klebsiella</i> spp. (60)				
Tigecycline	1	2	100.0	0.0
Tetracycline	4	>8	66.7	13.3
Imipenem	8	16	21.7	46.7
Meropenem	>8	>8	20.0	61.7
Ertapenem	>8	>32	5.0	83.3
Piperacillin-tazobactam	>64	>64	0.0	95.8
Cefepime	>16	>16	18.8	64.6
Aztreonam	>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 ^b	6.7 ^b
<i>Enterobacter</i> spp. (24)				
Tigecycline	0.25	0.5	100.0	0.0
Tetracycline	4	>8	70.8	20.8
Imipenem	4	>8	54.2	17.7
Meropenem	8	>8	33.3	41.7
Ertapenem	>8	>16	8.3	12.5
Piperacillin-tazobactam	32	>64	19.0	42.9
Cefepime	>16	>16	9.5	85.7
Aztreonam	>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 ^b	4.8 ^b

^a According to breakpoints established by the CLSI (4).

^b CLSI breakpoints for *P. aeruginosa* were applied for comparison purposes only.

Rates of susceptibility to other compounds varied from 0.0% for cefepime to 58.3% for gentamicin (Table 2). Tigecycline was the most active compound against carbapenemase-producing *Enterobacter* spp. (MIC₅₀, 0.25 µg/ml; MIC₉₀, 0.5 µg/ml; 100.0% susceptible), followed by amikacin and polymyxin B (95.2% susceptibility for both).

DISCUSSION

The widespread dissemination of carbapenemase-producing *Enterobacteriaceae* has profound implications for the clinical utility of the carbapenems (16). Furthermore, carbapenemase-producing *Enterobacteriaceae* strains were generally resistant to the vast majority of antimicrobial agents available for clinical use, making the therapeutic options very limited (6, 18). Fortunately, carbapenemase-producing *Enterobacteriaceae* strains are still extremely rare in most regions of the world; however, such isolates have been observed with great frequency in a few areas (15). For example, KPC-producing strains have become highly prevalent in the New York City area (2, 3, 8). The results of this study show that these enzymes have disseminated to other remote locations in the United

States. KPC-producing isolates were found in seven U.S. states, five of them located in the northeast area (including New York and nearby states); KPC was first detected in New York City (2). Additionally, these isolates demonstrated both intra- and interhospital clonal dissemination, mainly in the New York City area, confirming that this is a regional epidemic problem.

Similarly, M β L-producing *Enterobacteriaceae* have emerged in countries where M β L-producing *P. aeruginosa* strains have also become endemic, such as Greece, Turkey, Italy, and, more recently, Spain (15, 18). This suggests that *Enterobacteriaceae* isolates are likely to have acquired these enzyme-encoding genes either from the M β L-producing *P. aeruginosa* strains or from other nonfermentative species that could be the primary reservoir for M β L genetic elements.

Although clonal dissemination of carbapenemase-producing strains was observed in some medical centers, the high degree of genetic variability observed among carbapenemase-producing strains of the same species indicates that horizontal gene transfer is a major factor in the spread of these resistance mechanisms.

Approximately one-third of the studied strains had imipenem and/or meropenem MIC results within the CLSI (4) susceptible range despite carbapenemase production (Table 2). However, no randomized study evaluating the use of carbapenems for treatment of serine- or metallo- β -lactamase producing *Enterobacteriaceae* has been published; thus, the clinical usefulness of these antimicrobial agents under these conditions remains doubtful (6, 18) and in need of study by the CLSI.

All carbapenemase-producing *Enterobacteriaceae* isolates were inhibited at the tigecycline susceptibility breakpoint approved by the U.S. Food and Drug Administration (≤ 2 μ g/ml). This compound was the most active antimicrobial tested against this collection of multidrug-resistant strains (MIC₅₀, 0.5 μ g/ml; MIC₉₀, 2 μ g/ml) (9). However, it is important to note that tigecycline has not been approved for the treatment of bloodstream infections, and more clinical experience with this compound is necessary to better understand its role in the treatment of serious infections caused by carbapenemase-producing *K. pneumoniae* and other multidrug-resistant gram-negative bacilli.

The polymyxins (colistin and polymyxin B) can also be effective therapeutic alternatives, but the potential toxicity of these compounds and the need for association with another antimicrobial agent narrow their clinical use (6, 18).

This study, in addition to other recent surveillance initiatives (9–11), has determined that the antimicrobial activity of tigecycline is largely unaffected by mechanisms that most commonly occur in gram-negative organisms, such as extended-spectrum β -lactamase- and carbapenemase-medi-

ated resistance, confirming that this novel compound can be a valuable therapeutic option for the treatment of infections caused by these troublesome, resistant *Enterobacteriaceae*, as well as gram-positive cocci.

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