Prevalence and Antimicrobial Susceptibility Profile of ESBL- and KPC-producing Enterobacteriaceae in the United States (2003 – 2008)

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AMENDED ABSTRACT

Background: Appropriate empiric therapy for Enterobacteriaceae infections has become complex due to the high prevalence of several resistance mechanisms, including production of CTX-M and KPC enzymes. Among the limited therapeutic options, tigecycline has shown broad-spectrum activity against Gram-positive and -negative pathogens.

Methods: 6,277 *E. coli*, 3,762 *Klebsiella* spp. and 694 *P. mirabilis* were consecutively collected in USA centers during 2003 – 2008 and tested for susceptibility by CLSI methods (M07-A8, 2009). CLSI (M100-S20, 2010) and EUCAST (2010) interpretative criteria were applied. The US-FDA approved breakpoints for tigecycline were used. Isolates with elevated MIC values (≥2 μg/mL) for ceftazidime or ceftriaxone or aztreonam had the ESBL production confirmed. *E. coli* and *Klebsiella* spp. displaying MIC values ≥2 μg/mL for imipenem or meropenem were screened for bla_{KPC} by PCR and sequencing.

Results: ESBL rates among *E. coli* (ESBL-EC) increased from 3.0% in 2003 to 7.0% in 2008. Among *Klebsiella* spp., the ESBL rate (ESBL-KSP) increased from 9.8% in 2003 to 18.1% in 2005, decreasing to 14.4% in 2008. A great increase of ESBL-KSP in the New York City area skewed the overall rate in 2005. ESBL-producing *P. mirabilis* rates (ESBL-PM) varied from 1.1% in 2006 to 5.8% in 2007, remaining between 2.4 and 5.3% in other years. Tigecycline was the only agent with acceptable activity (≥90% susceptible) against ESBL-KSP (97.5% susceptible [CLSI]) and KPC-KSP (100.0% susceptible [CLSI]). Amikacin (MIC_{90}, 8 μg/mL; ≥90.0% susceptible), IMI (MIC_{90}, ≤0.06 μg/mL; ≥98.9% susceptible) and tigecycline (MIC_{90}, 0.25 μg/mL; ≥99.1% susceptible) were active against ESBL-EC, while other agents had limited activity (≤75.5% susceptible). ESBL-PM was very susceptible to amikacin (≥96.2%) and piperacillin/tazobactam (96.2%). Cefepime and levofloxacin exhibited suboptimal activity against ESBL-PM (73.1-88.5% susceptible). Tigecycline showed limited activity (30.8 – 61.5% susceptible) against ESBL-PM.

Antimicrobial Agent	ESBL-KSP (558)			ESBL-EC (351)			ESBL-PM (26)			KPC-KSP (90)		
	$MIC_{50/90}$	%S[a]	%S[b]	$MIC_{50/90}$	%S[a]	%S[b]	$MIC_{50/90}$	%S[a]	%S[b]	$MIC_{50/90}$	%S[a]	%S[b]
Amikacin	≤4/32	69.5	59.1	≤4/8	96.3	90.0	≤4/8	100.0	96.2	32/32	45.6	30.0
Cefepime	4/>16	60.9	30.5	1/>16	72.4	53.6	0.5/16	88.5	88.5	>16/>16	15.6	2.2
Imipenem	≤0.5/>8	75.5	74.0	≤0.06/≤0.06	99.7	98.9	2/4	100.0	88.5	>8/>8	8.9	3.3
Levofloxacin	>4/>4	37.3	34.1	>4/>4	35.3	34.2	≤0.5/4	80.8	73.1	>4/>4	22.2	17.8
P/T[c]	>64/>64	30.5	22.6	8/>64	75.5	63.8	≤0.5/2	96.2	96.2	>64/>64	2.2	2.2
Tigecycline	0.5/2	97.5	89.4	0.25/0.25	100.0	99.1	2/4	61.5	30.8	0.5/1	100.0	93.3

(a) CLSI and (b) EUCAST interpretation criteria, if available. (c) P/T = piperacillin/tazobactam. – = not available.

Conclusions: ESBL rates increased in the USA during the study interval. ESBL-producing Enterobacteriaceae, mainly *Klebsiella* spp., were generally multidrug-resistant (MDR). Tigecycline was active against *E. coli* and *Klebsiella* spp., regardless of resistance phenotype, and should be systematically evaluated for the treatment of infections caused by these MDR pathogens.

INTRODUCTION

Over the last decades an accumulation of extended-spectrum β-lactamase (ESBL)-encoding genes (bla_{TEM} and bla_{SHV}) has occurred among in *Escherichia coli* and *Klebsiella* spp. Concurrently, a generalized rise in fluoroquinolone-resistant organisms has been observed. However, more recently, a widespread dissemination of CTX-M enzymes has been reported in *E. coli*, *Klebsiella* spp. and other Enterobacteriaceae in the hospital and community environments.

Carbapenems are usually the therapeutic options when treating infections caused by ESBL-producing isolates. However, the clinical scenario has complicated further due to a worldwide spread of KPC-producing Enterobacteriaceae. In addition, metallo-β-lactamase producers (mainly VIM- and NDM-like) also present clinical significance in certain geographic regions. Such organisms are usually multidrug-resistant (MDR) and a rapid introduction of effective primary empiric broad-spectrum antimicrobial therapy is desirable.

Tigecycline was approved in the United States and Europe for the treatment of complicated skin and skin-structure infections (cSSSI) and intra-abdominal infections (IAI). Tigecycline possesses a proven broad-spectrum of activity against numerous bacterial pathogens, including aerobic and anaerobic species. In this study, we evaluated the activity and potency of tigecycline and comparator agents (18) tested against Enterobacteriaceae with documented ESBL or KPC production.

MATERIALS AND METHODS

Bacterial isolates. A total of 6,277 *E. coli*, 3,762 *Klebsiella* spp. and 694 *Proteus mirabilis* were consecutively collected in USA medical centers from 2003 to 2008. These clinical isolates were collected from hospitalized patients in a prevalence mode design, following specific protocols, and submitted to a central monitoring laboratory (JMI Laboratories, North Liberty, Iowa) as part of the SENTRY Antimicrobial Surveillance Program. Bacterial species identifications were confirmed using standard algorithms and the automated Vitek 2 system (bioMérieux, Hazelwood, Missouri, USA).

Antimicrobial susceptibility testing. Isolates were tested for susceptibility by reference broth microdilution methods using the Clinical Laboratory Standards Institute (CLSI) recommendations (M07-A8, 2009). Susceptibility testing was performed by using validated broth microdilution panels manufactured by TREK Diagnostics Systems/Sensititre (Cleveland, Ohio, USA).

Validation of the minimum inhibitory concentration (MIC) values was performed by concurrent testing of CLSI-recommended (M100-S20, 2010) quality control (QC) strains: *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. MIC ranges for tigecycline and comparator agents tested against ATCC QC strains were those published in the CLSI M100-S20 (2010) document.

Interpretations of comparator MIC values were performed using the CLSI (M100-S20, 2010) and European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2010) criteria, when available. Tigecycline breakpoints approved by the US-FDA for Enterobacteriaceae (≤2 μg/mL for susceptible and ≥8 μg/mL for resistant) were utilized.

Confirmation of ESBL and KPC production. Isolates with elevated MIC values (≥2 μg/mL) for ceftazidime or ceftriaxone or aztreonam were selected for further confirmation of the ESBL production, which was performed using the disk approximation method or Etest (bioMerieux, Inc., Durham, North Carolina, USA) according to the manufacture's instruction. *Klebsiella* spp. and *E. coli* displaying MIC values at ≥2 μg/mL for imipenem or meropenem were screened for bla_{KPC} by PCR and sequencing.

ACKNOWLEDGMENT

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RESULTS

- Among *Klebsiella* spp. isolates, the ESBL rates increased from 9.8% in 2003 to 18.1% in 2005 due to a higher number of ESBL strains from the New York City area. The following years had stable rates around 15% (Figure 1).

Table 1. Activity of tigecycline and comparator agents tested against ESBL-producing Enterobacteriaceae clinical isolates collected as part of the SENTRY Antimicrobial Surveillance Program (2003 - 2008).

Organism (number tested)/ Antimicrobial agent	MIC (μg/mL)		Range	% susceptible / % resistant[a]	
	50%	90%		CLSI	EUCAST
E. coli (351)					
Tigecycline	0.25	0.25	0.06 – 2	100.0[b] / 0.0[b]	99.1 / 0.0
Aztreonam	8	>16	≤0.12 – >16	40.7 / 41.6	12.8 / 41.6
Ceftriaxone	16	>32	≤0.25 – >32	26.8 / 68.4	26.8 / 68.4
Ceftazidime	16	>16	≤1 – >16	40.5 / 50.4	15.1 / 50.4
Ampicillin	>16	>16	≤1 – >16	2.3 / 97.7	- / 97.7
Ampicillin/sulbactam	>16	>16	≤2 – >16	5.7 / 68.7	- / 94.3
Cefepime	1	>16	≤0.12 – >16	72.4 / 22.5	53.6 / 27.6
Ertapenem	≤0.06	0.25	≤0.06 – >8	98.3 / 1.7	96.2 / 1.7
Imipenem	≤0.5	≤0.5	≤0.5 – >8	99.7 / 0.3	98.9 / 0.3
Meropenem	≤0.12	≤0.12	≤0.12 – >8	99.7 / 0.3	98.6 / 0.3
Piperacillin/tazobactam	8	>64	≤0.5 – >64	75.5 / 11.1	63.8 / 24.5
Ciprofloxacin	>4	>4	≤0.03 – >4	34.9 / 64.6	34.3 / 65.1
Levofloxacin	>4	>4	≤0.5 – >4	35.3 / 62.4	34.2 / 64.7
Amikacin	≤4	8	≤4 – >32	96.3 / 1.4	90.0 / 3.7
Gentamicin	≤2	>8	≤2 – >8	67.0 / 30.2	63.5 / 33.0
Tobramycin	2	>16	≤0.25 – >16	60.7 / 30.2	53.6 / 39.3
Tetracycline	>8	>8	≤2 – >8	39.3 / 59.5	- / -
Trimethoprim/sulfamethoxazole	2	>2	≤0.5 – >2	50.7 / 49.3	50.7 / 49.3
P. mirabilis (26)					
Tigecycline	2	4	0.5 – 4	61.5[b] / 0.0[b]	30.8 / 38.5
Aztreonam	0.25	>16	≤0.12 – >16	88.5 / 11.5	65.4 / 11.5
Ceftriaxone	2	32	≤0.25 – >32	42.3 / 19.2	42.3 / 19.2
Ceftazidime	2	8	≤1 – >16	84.6 / 7.7	38.5 / 7.7
Ampicillin	>16	>16	≤1 – >16	26.9 / 73.1	- / 73.1
Ampicillin/sulbactam	16	>16	≤2 – >16	42.3 / 26.9	- / 57.7
Cefepime	0.5	16	≤0.12 – >16	88.5 / 7.7	88.5 / 11.5
Ertapenem	≤0.06	≤0.06	≤0.06 – 2	100.0 / 0.0	96.2 / 3.8
Imipenem	2	4	≤0.5 – 4	100.0 / 0.0	88.5 / 0.0
Meropenem	≤0.12	≤0.12	≤0.12 – 4	100.0 / 0.0	96.2 / 0.0
Piperacillin/tazobactam	≤0.5	2	≤0.5 – 32	96.2 / 0.0	96.2 / 3.8
Ciprofloxacin	0.12	>4	≤0.03 – >4	69.2 / 15.4	53.8 / 30.8
Levofloxacin	≤0.5	>4	≤0.5 – >4	80.8 / 19.2	73.1 / 19.2
Amikacin	≤4	8	≤4 – 16	100.0 / 0.0	96.2 / 0.0
Gentamicin	≤2	>8	≤2 – >8	65.4 / 19.2	57.7 / 34.6
Tobramycin	1	16	0.25 – >16	76.9 / 11.5	61.5 / 23.1
Tetracycline	>8	>8	≤2 – >8	3.8 / 96.2	- / -
Trimethoprim/sulfamethoxazole	2	>2	≤0.5 – >2	50.0 / 50.0	50.0 / 50.0
Klebsiella spp.[d] (558)					
Tigecycline	0.5	2	0.12 – 4	97.5[b] / 0.0[b]	90.0 / 2.5
Aztreonam	>16	>16	≤0.12 – >16	18.3 / 77.4	10.8 / 77.4
Ceftriaxone	32	>32	≤0.25 – >32	9.9 / 85.1	9.9 / 85.1
Ceftazidime	>16	>16	≤1 – >16	25.8 / 71.5	14.3 / 71.5
Ampicillin	>16	>16	4 – >16	0.2 / 99.6	- / 99.8
Ampicillin/sulbactam	>16	>16	≤2 – >16	4.5 / 84.2	- / 95.5
Cefepime	4	>16	≤0.12 – >16	60.9 / 29.0	30.5 / 39.1
Ertapenem	≤0.06	>8	≤0.06 – >8	72.4 / 26.1	69.9 / 28.9
Imipenem	≤0.5	>8	≤0.5 – >8	75.5 / 19.9	74.0 / 19.9
Meropenem	≤0.12	>8	≤0.12 – >8	76.0 / 20.3	73.7 / 20.3
Piperacillin/tazobactam	>64	>64	≤0.5 – >64	30.5 / 59.9	22.6 / 69.5
Ciprofloxacin	>4	>4	≤0.03 – >4	34.1 / 62.5	29.7 / 65.9
Levofloxacin	>4	>4	≤0.5 – >4	37.3 / 57.9	34.1 / 62.7
Amikacin	≤4	32	≤4 – >32	69.5 / 6.1	59.1 / 30.5
Gentamicin	4	>8	≤2 – >8	53.8 / 34.8	46.4 / 46.2
Tobramycin	16	>16	≤0.25 – >16	32.3 / 57.0	26.0 / 67.7
Tetracycline	≤4	>8	≤4 – >8	63.4 / 26.3	- / -
Trimethoprim/sulfamethoxazole	>2	>2	≤0.5 – >2	30.9 / 69.1	30.9 / 69.1

a. Criteria as published by the CLSI (2010) and EUCAST (2010).
b. US-FDA breakpoints were applied (Tygacil Product Insert, 2005).
c. Breakpoint not available.
d. Includes: *K. oxytoca* (48 strains), *K. ozaenae* (1 strain), *K. pneumoniae* (842 strains), and unspeciated *Klebsiella* (1 strain).

- ESBL rates among *E. coli* increased from 3.0% in 2003 to 7.0% in 2008. ESBL-producing *P. mirabilis* rates varied from 1.1% in 2006 to 5.8% in 2007, remaining between 2.4 and 5.3% in other years (Figure 1).
- ESBL-producing *E. coli* were very susceptible to tigecycline ($MIC_{50/90}$, 0.25/0.25 μg/mL; ≥99.1% susceptible; Table 1). Only carbapenems (ertapenem, imipenem and meropenem) and amikacin (≥90.0% susceptible) showed antimicrobial coverage against these isolates.
- Tigecycline ($MIC_{50/90}$, 2/4 μg/mL; 30.8 – 61.5% susceptible) was less active against ESBL-producing *P. mirabilis* (Table 1). Cephalosporin agents demonstrated limited activity against these strains (38.5 – 88.5% susceptible). Whereas, carbapenems and amikacin (100.0% susceptible [CLSI]), and piperacillin/tazobactam (96.2% susceptible) were also active against ESBL-producing *P. mirabilis.*

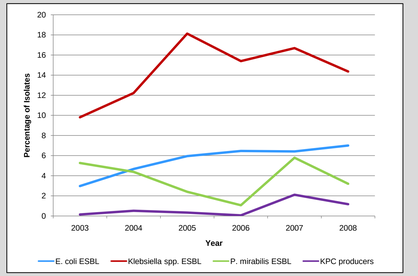
Table 2. Activity of tigecycline and comparator agents tested against 90 KPC-producing *Klebsiella* spp.[a] clinical isolates collected as part of the SENTRY Antimicrobial Surveillance Program (2003 - 2008).

Antimicrobial agent	MIC (μg/mL)		Range	% susceptible / % resistant[b]	
	50%	90%		CLSI	EUCAST
Tigecycline	0.5	1	0.12 – 2	100.0[c] / 0.0[c]	93.3 / 0.0
Aztreonam	>16	>16	≤0.12 – >16	1.1 / 97.8	1.1 / 97.8
Ceftriaxone	>32	>32	≤0.25 – >32	1.1 / 98.9	1.1 / 98.9
Ceftazidime	>16	>16	≤1 – >16	5.6 / 92.2	3.3 / 92.2
Ampicillin	>16	>16	16 – >16	0.0 / 98.9	-[d] / 100.0
Ampicillin/sulbactam	>16	>16	≤2 – >16	2.2 / 97.8	- / 97.8
Cefepime	>16	>16	≤0.12 – >16	15.6 / 65.6	2.2 / 84.4
Ertapenem	>8	>8	≤0.06 – >8	3.4 / 89.8	1.1 / 98.9
Imipenem	>8	>8	0.5 – >8	8.9 / 70.0	3.3 / 70.0
Meropenem	>8	>8	≤0.12 – >8	13.3 / 70.0	3.3 / 70.0
Piperacillin/tazobactam	>64	>64	1 – >64	2.2 / 95.6	2.2 / 97.8
Ciprofloxacin	>4	>4	≤0.03 – >4	16.7 / 80.0	13.3 / 83.3
Levofloxacin	>4	>4	≤0.5 – >4	22.2 / 76.7	17.8 / 77.8
Amikacin	32	32	0.5 – >32	45.6 / 5.6	30.0 / 54.4
Gentamicin	4	>8	≤2 – >8	50.0 / 35.6	40.0 / 50.0
Tobramycin	>16	>16	0.25 – >16	7.8 / 88.9	6.7 / 92.2
Tetracycline	≤4	>8	≤4 – >8	72.2 / 11.1	- / -
Trimethoprim/sulfamethoxazole	>2	>2	≤0.5 – >2	8.9 / 91.1	8.9 / 91.1

a. Includes: *K. oxytoca* (10 strains), and *K. pneumoniae* (80 strains).
b. Criteria as published by the CLSI (2010) and EUCAST (2010).
c. US-FDA breakpoints were applied (Tygacil Product Insert, 2005).
d. Breakpoint not available.

- Except for tigecycline (≥90.0% susceptible), other antimicrobial agents exhibited limited activity (≤76.0% susceptible) against ESBL-producing *Klebsiella* spp. (Table 1).
- Only tigecycline ($MIC_{50/90}$, 0.5/1 μg/mL; ≥93.3.0% susceptible) demonstrated antimicrobial activity against KPC-producing *Klebsiella* spp. (Table 2). Comparator agents showed elevated resistance rates (5.6 – 100.0%).

Figure 1. Rates of ESBL- and KPC-producing Enterobacteriaceae collected from USA hospitals during 2003 – 2008, as part of the SENTRY Antimicrobial Surveillance Program.



CONCLUSIONS

- Rates of ESBL producers increased among USA clinical isolates during the study interval. Increased rates were more significant among *Klebsiella* spp. strains.
- Tigecycline and carbapenems demonstrated comparable activity when tested against ESBL-producing *E. coli*. However, only tigecycline exhibited potent activity against KPC-producing *Klebsiella* spp.
- The potent tigecycline activity obtained against KPC-producing *Klebsiella* spp. warrants systematic evaluation for the treatment of infections caused by these MDR pathogens.