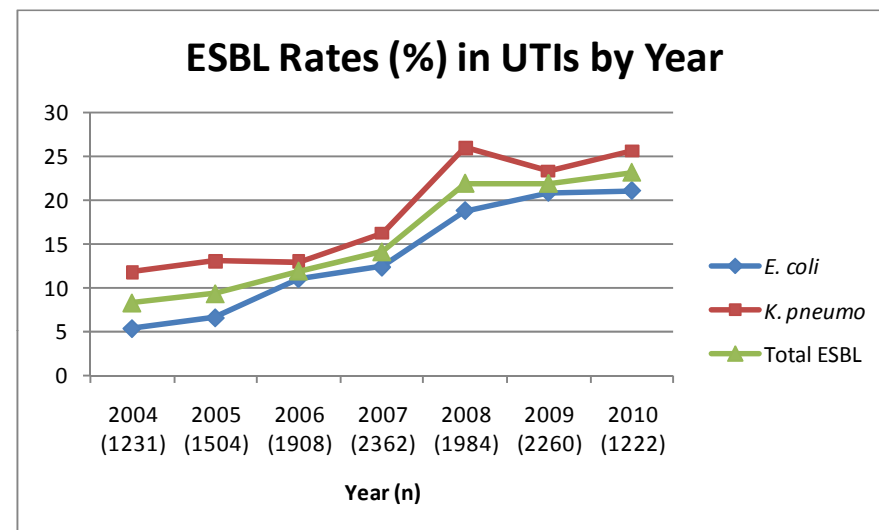


Revised Abstract

Background: *Escherichia coli* and *Klebsiella pneumoniae* are major pathogens in the etiology of urinary tract infections (UTIs). The treatment of these species has been complicated with increasing rates of extended spectrum beta-lactamase (ESBL) production in recent years. This study looks at the trends in ESBL production from 2004 through 2010 and the effect on susceptibilities to common therapeutic agents. The Tigecycline Evaluation and Surveillance Trial (TEST) is an ongoing global surveillance designed to follow trends in antimicrobial activity of tigecycline and comparators. **Methods:** 12,471 clinical isolates were collected from 525 investigator sites globally. Collection of isolates was limited to clinical UTI sources. Clinical isolates were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using sponsor supplied broth microdilution panels and interpreted according to current CLSI and FDA (tigecycline) guidelines. **Results:** Tigecycline *in vitro* activity has remained stable since 2004 with MIC₅₀, MIC₉₀, and %Sus in 2009/2010 of 0.25 mcg/ml, 1 mcg/ml, and 99.8%, respectively for ESBL+ *E. coli*, and 1 mcg/ml, 4 mcg/ml, and 89.8% for ESBL+ *K. pneumoniae*. Regional ESBL rates varied from a low rate of 10% in North America to 31% in Asia for *E. coli*, and from 9% in North America to 31% in Europe for *K. pneumoniae*. Global trends in ESBL rates are presented in the following figure:



Conclusions: Overall ESBL production in *E. coli* and *K. pneumoniae* from UTIs has shown a steady rise worldwide during the seven year period of this study (p<0.0001) with wide variations demonstrated regionally. Tigecycline *in vitro* activity against ESBL producing *E. coli* and *K. pneumoniae* against UTI isolates has not changed significantly since its introduction in 2005 (p>0.05) with susceptibilities of about 100% and 90%, respectively, in 2009/2010.

Introduction

Escherichia coli and *Klebsiella pneumoniae* are major pathogens in the etiology of urinary tract infections (UTIs). The treatment of these species has been complicated with increasing rates of extended spectrum beta-lactamase (ESBL) production in recent years. First detected in the mid-1980's, proliferation of ESBLs has become a serious global health concern, with the prevalence varying greatly from country to country.

Tigecycline is very active *in vitro* against ESBL producing *Enterobacteriaceae*, however it reaches only low urinary concentrations. With only rare published accounts of successful UTI treatment with tigecycline when other treatments failed [1,2], there is currently no FDA clinical indication for tigecycline in UTIs, and the routine use for UTIs when other viable treatment options are available is not recommended [3,4]. The Tigecycline Evaluation and Surveillance Trial (TEST) is an ongoing global surveillance study designed to follow trends in antimicrobial activity of tigecycline and comparators. This report focuses on worldwide trends in ESBL production from 2004 through 2010 and the effect on susceptibilities to tigecycline and common therapeutic agents.

Materials & Methods

- Between 2004 and 2010, 525 sites in 61 countries collected 7,116 isolates of *E. coli* and 5,355 isolates of *K. pneumoniae* from the urinary tract. Only one isolate per patient was accepted into the study. Isolates were identified to the species level and MICs determined at each participating laboratory using sponsor supplied broth microdilution panels.
- Organism collection, transport, confirmation of organism identification, and development and management of a centralized database were coordinated by Laboratories International for Microbiology Studies (LIMS), a division of International Health Management Associates, Inc. located in Schaumburg, IL, USA.
- Minimum inhibitory concentrations (MICs) were determined by the Clinical and Laboratory Standards Institute (CLSI) recommended broth microdilution testing method using MicroScan (Siemens Medical Solutions Diagnostics, West Sacramento, CA) or Sensititre (TREK Diagnostic Systems, Cleveland, OH) panels [5]. All antimicrobics were supplied by the panel manufacturers.
- MIC interpretive criteria followed published guidelines of the CLSI and the most recent United States Food and Drug Administration (FDA) package insert for tigecycline where applicable [6,7].
- Isolates were screened for extended spectrum beta-lactamase production by broth microdilution and confirmed using the disc diffusion confirmatory test [6].
- Quality controls (QC) were performed on each day of testing using appropriate ATCC control strains, following CLSI and manufacturer guidelines. Results were included in the analysis only when corresponding QC results were within the acceptable ranges [6].
- Confidence intervals were calculated using the adjusted Wald method. Differences in percent susceptible between regions and the worldwide average were evaluated for significance using Fisher's exact test. The Cochran-Armitage test was used to assess linear trends in percent susceptible over time.

References

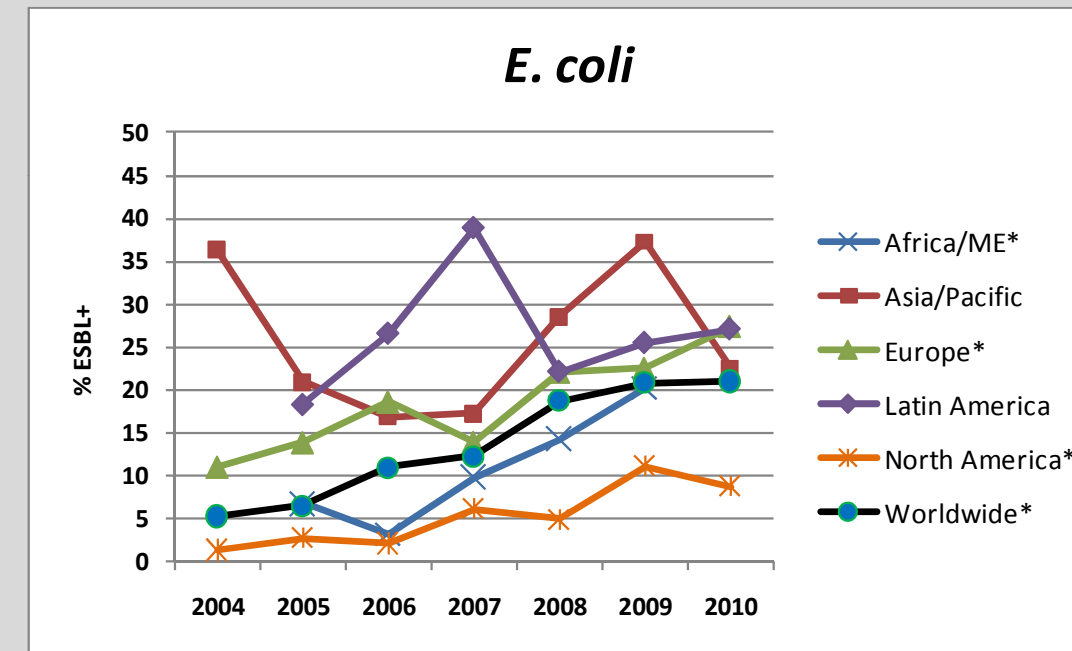
- Krueger WA, Kempt VA, Peiffer M, et al. *Treatment with tigecycline of recurrent urosepsis caused by extended-spectrum-β-lactamase-producing Escherichia coli*. J Clin Microbiol. 2008;46(2):817–820.
- Geerlings SE, van Donselaar-van der Pant KA, Keur I. *Successful treatment with tigecycline of two patients with complicated urinary tract infections caused by extended-spectrum beta-lactamase-producing Escherichia coli*. J Antimicrob Chemother. 2010 Sep;65(9):2048-9. Epub 2010 Jun 16.
- Bader MS, Hawboldt J, Brooks A. *Management of complicated urinary tract infections in the era of antimicrobial resistance*. Postgrad Med. 2010 Nov;122(6):7-15. PMID: 21084776.
- Nix DE, Matthias KR. *Should tigecycline be considered for urinary tract infections? A pharmacokinetic re-evaluation*. J Antimicrob Chemother. 2010 Jun;65(6):1311-2. Epub 2010 Apr 8. PMID: 20378673.
- Clinical Laboratory Standards Institute. 2009. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standards – 8th Edition. CLSI document M07-A8. Wayne, PA.
- Clinical and Laboratory Standards Institute. 2011. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. CLSI Document M100-S21. Wayne, PA.
- Tygacil®, 2011. Tigecycline FDA prescribing information. Pfizer, Inc., Collegeville, PA.

Acknowledgements

We gratefully acknowledge the contributions of the investigators, laboratory personnel, and all members of the Tigecycline Evaluation and Surveillance Trial group. This study was sponsored by Pfizer Inc. IHMA is a clinical research organization that has been contracted by Pfizer Inc. to manage the TEST program.

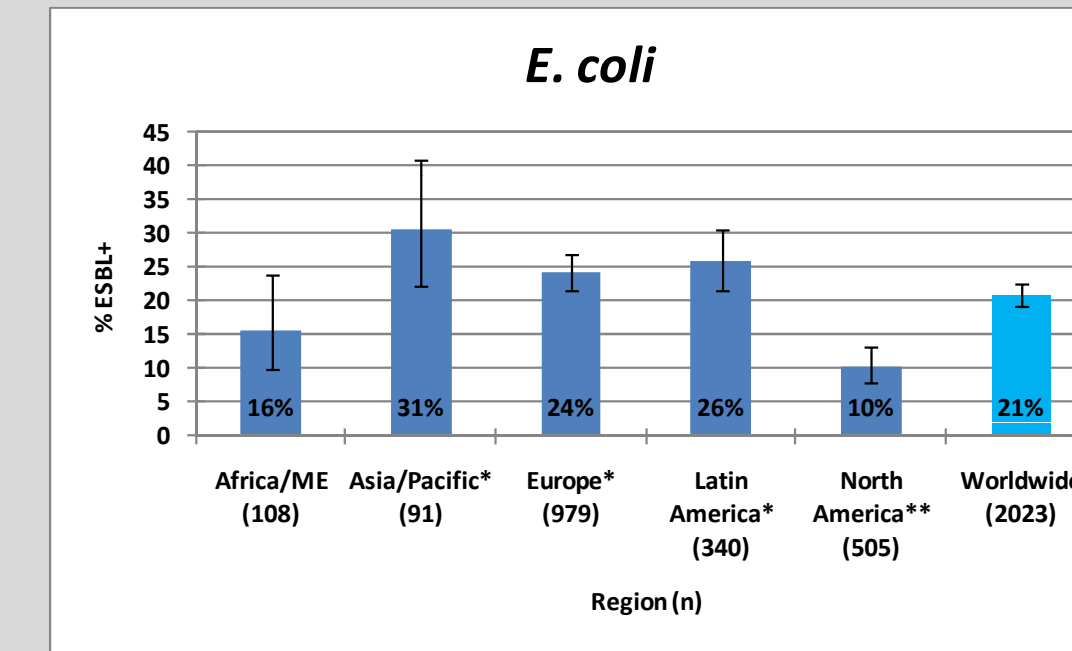
Results

Figure 1. Trends in ESBL+ rates for *E. coli* by region (for all contiguous years with n≥30).



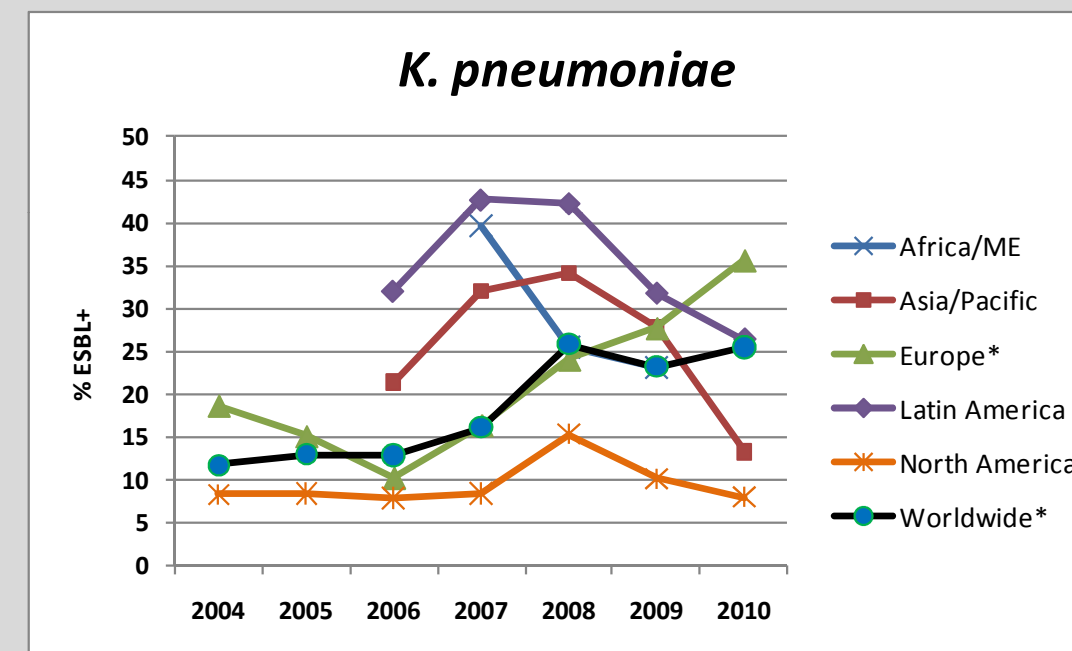
* Statistically significant increasing trend (p<0.05). ME - Middle East.
 n per year: Africa/ME 0/73/62/61/49/84/24, Asia/Pacific 33/43/95/104/56/51/40, Europe 153/129/263/336/588/625/354, Latin America 9/49/154/126/216/270/70, North America 483/579/507/704/221/323/182, Worldwide 678/873/1081/1331/1130/1353/670.

Figure 3. ESBL+ rates for 2,023 recent *E. coli* isolates by region, 2009/2010 (with 95% confidence interval).



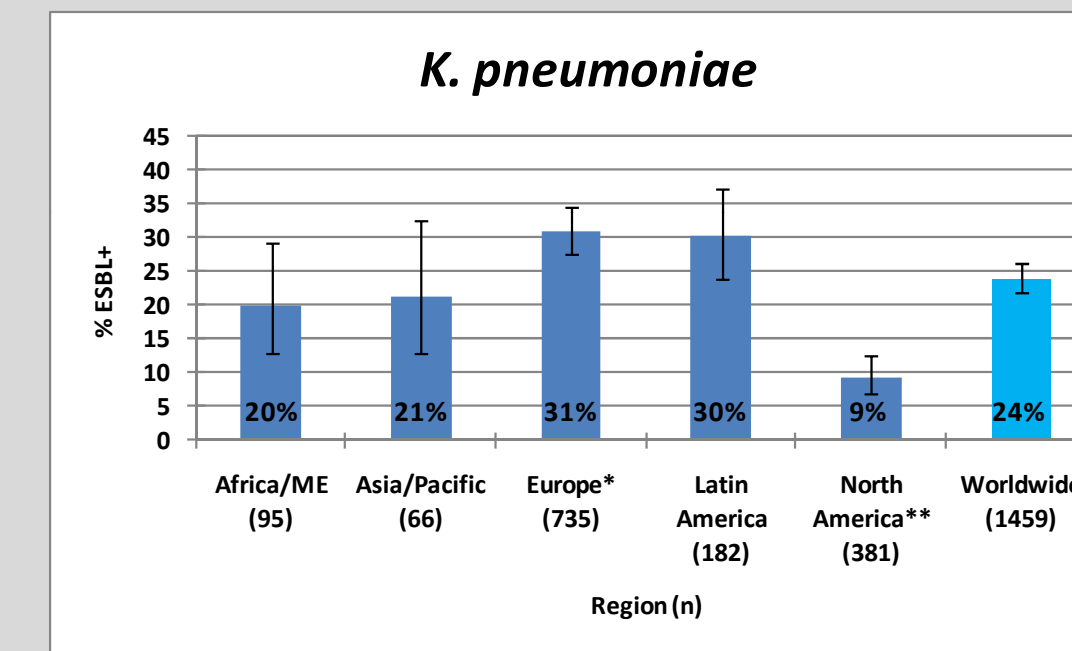
* % ESBL+ is significantly higher than the worldwide average (p<0.05).
 ** % ESBL+ is significantly lower than the worldwide average (p<0.05).
 ME - Middle East.

Figure 2. Trends in ESBL+ rates for *K. pneumoniae* by region (for all contiguous years with n≥30).



* Statistically significant increasing trend (p<0.05). ME - Middle East.
 n per year: Africa/ME 0/42/15/53/51/69/26, Asia/Pacific 29/14/42/78/41/36/30, Europe 128/79/203/237/444/429/306, Latin America 13/26/100/75/142/129/53, North America 383/470/467/588/176/244/137, Worldwide 553/631/827/1031/854/507/552.

Figure 4. ESBL+ rates for 1,459 recent *K. pneumoniae* isolates by region, 2009/2010 (with 95% confidence interval).



* % ESBL+ is significantly higher than the worldwide average (p<0.05).
 ** % ESBL+ is significantly lower than the worldwide average (p<0.05).
 ME - Middle East.

Conclusions

- Overall ESBL production in *E. coli* and *K. pneumoniae* from UTIs has shown a steady rise worldwide during the seven year period of this study (p<0.0001) with wide variations demonstrated regionally. Significantly higher ESBL+ rates were found for recent isolates in Asia/Pacific, Europe, and Latin America, while rates in North America were significantly lower than the global average (p<0.05).
- Tigecycline's *in vitro* susceptibility against ESBL producing *E. coli* and *K. pneumoniae* from UTIs has not changed significantly since its introduction in 2005 (p>0.05) with susceptibilities of about 100% and 90%, respectively, in 2009/2010.
- Similar susceptibilities were found only for amikacin and meropenem, with all other studied antimicrobials showing much weaker activity, especially against ESBL+ *K. pneumoniae*.
- Although this study reports on the potent *in vitro* activity of tigecycline against selected UTI pathogens, there is no FDA clinical indication for UTIs.
- Additional studies on the activity of tigecycline in UTIs are warranted as well as vigilant monitoring of ESBL prevalence to help determine the most effective choice of therapy especially in regions with high and/or increasing rates of ESBL+ isolates.

Table 1. Trends in *in vitro* activity of tigecycline against 1,996 ESBL+ *E. coli* and *K. pneumoniae* isolates from the urinary tract, 2004-2010.

Year	<i>E. coli</i> , ESBL+				<i>K. pneumoniae</i> , ESBL+			
	n	MIC ₅₀	MIC ₉₀	%S ¹	n	MIC ₅₀	MIC ₉₀	%S ¹
2004	36	0.25	0.5	100	62	0.5	4	83.9
2005	57	0.25	0.5	100	76	1	2	90.8
2006	119	0.25	0.5	100	107	1	4	89.7
2007	164	0.25	0.5	99.4	167	1	2	94.0
2008	212	0.25	1	100	221	1	2	91.0
2009	282	0.25	1	99.7	211	1	2	90.5
2010	141	0.25	0.5	100	141	1	4	88.7

¹%S - % susceptible. No statistically significant trends in %S were found (p>0.05).

Table 2. *In vitro* activity of tigecycline and comparators against 3,482 recent *E. coli* and *K. pneumoniae* isolates from the urinary tract, 2009/2010.

Organism	Drug	MIC ₅₀	MIC ₉₀	%S	%I	%R
<i>E. coli</i> (n=2023)						
	Amikacin	2	8	98.0	1.2	0.8
	Amox-Clav	8	32	59.6	23.0	17.4
	Ampicillin	>32	>32	30.0	1.3	68.8
	Cefepime	≤0.5	>32	82.7	3.0	14.3
	Ceftriaxone	0.12	>64	67.9	1.4	30.7
	Levofloxacin	0.5	>8	56.6	1.5	41.9
	Meropenem	≤0.06	≤0.06	99.3	0.4	0.4
	Minocycline	2	16	67.6	12.2	20.2
	Pip-Tazo	2	32	87.3	7.6	5.1
	Tigecycline	0.25	0.5	99.9	0.1	0
<i>E. coli</i> , ESBL+ (n=423)						
	Amikacin	4	16	94.6	3.6	1.9
	Amox-Clav	16	32	27.7	44.0	28.4
	Ampicillin	>32	>32	0.2	0	99.8
	Cefepime	32	>32	29.8	10.6	59.6
	Ceftriaxone	>64	>64	1.0	0.7	98.4
	Levofloxacin	>8	>8	17.3	1.7	81.1
	Meropenem	≤0.06	≤0.06	98.8	1.0	0.2
	Minocycline	4	>16	55.8	17.7	26.5
	Pip-Tazo	8	64	72.1	18.0	9.9
	Tigecycline	0.25	1	99.8	0.2	0
<i>K. pneumoniae</i> (n=1459)						
	Amikacin	1	8	94.3	2.4	3.3
	Amox-Clav	4	32	61.8	14.7	23.6
	Ampicillin	>32	>32	1.4	8.8	89.9
	Cefepime	≤0.5	>32	77.6	3.8	18.6
	Ceftriaxone	0.12	>64	64.5	1.4	34.1
	Levofloxacin	0.12	>8	69.2	4.4	26.4
	Meropenem	≤0.06	0.25	96.1	0.9	3.0
	Minocycline	4	>16	57.4	15.8	26.8
	Pip-Tazo	4	>128	73.6	9.1	17.3
	Tigecycline	0.5	2	95.0	4.0	1.0
<i>K. pneumoniae</i> , ESBL+ (n=352)						
	Amikacin	4	64	83.8	6.0	10.2
	Amox-Clav	16	>32	14.8	36.9	48.3
	Ampicillin	>32	>32	0	0.3	99.7
	Cefepime	32	>32	29.0	11.7	59.4
	Ceftriaxone	>64	>64	1.1	0.6	98.3
	Levofloxacin	>8	>8	23.3	9.1	67.6
	Meropenem	≤0.06	1	92.3	1.7	6.0
	Minocycline	8	>16	27.0	23.0	50.0
	Pip-Tazo	64	>128	37.8	21.9	40.3
	Tigecycline	1	4	89.8	7.7	2.6