

Revised Abstract

Objective: Carbapenemase-producing *Enterobacteriaceae* are spreading globally. They are often resistant to all β -lactam antibiotics and co-resistant to most other antibiotics, leaving very few treatment options. Using surveillance data from the global, comprehensive Tigecycline Evaluation and Surveillance Trial (TEST), we assessed changes in incidence and susceptibility of meropenem non-susceptible *Enterobacteriaceae* between the years 2006/2007 and 2009/2010.

Methods: A total of 1,218 meropenem non-susceptible *Enterobacteriaceae* were isolated in 46 countries from multiple infection sources. MICs were determined by each participating laboratory using commercially-prepared broth microdilution panels. Results were interpreted according to CLSI breakpoints (including new meropenem breakpoints published in June 2010) or FDA breakpoints (for tigecycline). The difference in % susceptible values between 2006/2007 and 2009/2010 was tested using the chi square test, while the geometric mean MICs were compared by t-test of log-transformed MICs.

Results: 626 meropenem non-susceptible *Enterobacteriaceae* were collected in 2006/2007 and 592 isolates in 2009/2010. These represented 2.1% and 2.6% of all *Enterobacteriaceae*, respectively -- a significant increase ($p < 0.0001$) from 2006/2007 to 2009/2010. The following table shows the MIC₅₀, geometric mean (GM) MIC, and % susceptible for tigecycline and several comparators, as well as p values comparing both time periods.

Drug	MIC ₅₀		GM MIC		p*	%Susceptible	
	2006/07	2009/10	2006/07	2009/10		2006/07	2009/10
Tigecycline	1	1	0.8	1.0	<0.0001	90.7	84.5
Amikacin	16	16	13.8	13.4	0.73	62.6	60.3
Cefepime	32	32	19.7	16.3	0.03	28.6	34.5
Levofloxacin	>8	8	4.3	4.0	0.55	29.4	32.3
Minocycline	4	8	6.1	9.7	<0.0001	50.8	29.1
Pip-Tazo	>128	>128	67.9	73.1	0.46	25.4	0.39

* Testing the difference between 2006/2007 and 2009/2010; boldface indicates statistical significance ($p < 0.05$)

Conclusions: While the MIC₅₀ for tigecycline did not change between 2006/2007 and 2009/2010, the geometric mean increased significantly, demonstrating that this measure may offer better discrimination. The increase in GM MIC for minocycline was also significant, while the decrease for cefepime was only marginally so. Despite tigecycline's increase in GM MIC and a statistically significant decrease in % susceptible, the agent continued to demonstrate the best *in vitro* activity against meropenem non-susceptible *Enterobacteriaceae*. Almost 85% of these usually multi-resistant pathogens remained susceptible to tigecycline, confirming tigecycline as an important therapeutic option, especially since the other antimicrobials exhibited much poorer *in vitro* activity.

Introduction

Increased spread of extended-spectrum β -lactamases (ESBLs) among members of the family *Enterobacteriaceae* has resulted in an increase in the use of carbapenems. Carbapenems are among the few therapies available for serious infections caused by multidrug-resistant gram-negative bacteria. However, carbapenemase-producing *Enterobacteriaceae* are spreading globally. They are often resistant to all β -lactam antibiotics and co-resistant to most other antibiotics, leaving very few treatment options. Using surveillance data from the global, comprehensive Tigecycline Evaluation and Surveillance Trial (TEST), we assessed changes in incidence and susceptibility of meropenem non-susceptible *Enterobacteriaceae* between the years 2006/2007 and 2009/2010 as well as differences in incidence and susceptibility of recent isolates across regions and species.

Materials & Methods

In 2006/2007 and 2009/2010, 1,218 meropenem non-susceptible (MIC > 1mg/L) *Enterobacteriaceae* (*Enterobacter* spp., *Escherichia coli*, *Klebsiella* spp., and *Serratia* spp.) were collected globally from 247 medical centers in 46 countries. Isolates were derived from blood (22%), respiratory tract (21%), urine (19%), bodily fluids (13%), wounds (10%), and various other infection sources. Only one isolate per patient was accepted into the study. Isolates were identified to the species level and MICs determined at each site by the participating laboratory.

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 [1]. Tigecycline was supplied by Pfizer, Inc. (Collegetown, PA, USA). All other agents were supplied by the panel manufacturers MicroScan (Siemens Medical Solutions Diagnostics., West Sacramento, CA, USA) and TREK (TREK Diagnostic Systems, Cleveland, OH).

Quality control (QC) of broth microdilution panels followed manufacturers and CLSI guidelines using *E. coli* ATCC 25922, *E. coli* ATCC 35218, and *P. aeruginosa* ATCC 27853. Results were included in the analysis only when QC isolates tested within the acceptable range according to CLSI guidelines [2].

MIC interpretive criteria followed published breakpoints defined by CLSI [2] and the United States Food and Drug Administration (FDA) package insert for tigecycline [3].

Geometric mean MICs were compared by t-test of log-transformed MICs and proportions of meropenem non-susceptible by the chi square test. A p value < 0.05 was considered statistically significant.

References

- Clinical Laboratory Standards Institute. 2009. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standards -- Eighth 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®. 2010. Tigecycline FDA prescribing information. Pfizer, Inc., Collegetown, PA.

Acknowledgements

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Results

Globally, 2.1% of all *Enterobacteriaceae* collected in 2006/2007 (626 of 29,495) were meropenem non-susceptible, while 2.6% (592 of 22,375) were meropenem non-susceptible in 2009/2010. These data represent a statistically significant increase ($p < 0.0001$) from 2006/2007 to 2009/2010. Tables 1 and 2 describe differences in *in vitro* activity and percent susceptibility for both time periods. The remaining tables and figures describe in more detail characteristics of the more recent isolates derived from 2009/2010.

Table 1. *In vitro* activity of tigecycline and comparators against meropenem non-susceptible *Enterobacteriaceae* globally, 2006/2007 versus 2009/2010.

Drug	2006/07 (n=626)			2009/10 (n=592)			p*
	MIC ₅₀	MIC ₉₀	GM MIC	MIC ₅₀	MIC ₉₀	GM MIC	
	Tigecycline	1	2	0.8	1	4	
Amikacin	16	> 64	13.8	16	> 64	13.4	0.73
Cefepime	32	> 32	19.7	32	> 32	16.3	0.03
Levofloxacin	> 8	> 8	4.3	8	> 8	4.0	0.55
Minocycline	4	> 16	6.1	8	> 16	9.7	<0.0001
Pip-Tazo	> 128	> 128	67.9	> 128	> 128	73.1	0.46

GM: geometric mean; MIC in mg/L

* Testing the difference in GM MIC between 2006/2007 and 2009/2010; boldface indicates statistical significance ($p < 0.05$)

Table 2. Susceptibility of tigecycline and comparators against meropenem non-susceptible *Enterobacteriaceae* globally, 2006/2007 versus 2009/2010.

Drug	2006/07 (n=626)			2009/10 (n=592)			p*
	%S	%I	%R	%S	%I	%R	
Tigecycline	90.7	8.2	1.1	84.5	10.0	5.6	0.001
Amikacin	62.6	14.1	23.3	60.1	10.8	29.1	0.41
Cefepime	28.6	11.3	60.1	34.5	13.2	52.4	0.03
Levofloxacin	29.4	7.4	63.3	32.1	9.5	58.5	0.29
Minocycline	50.8	20.0	29.2	29.2	24.0	46.8	<0.0001
Pip-Tazo	25.4	11.3	63.3	23.3	16.9	59.8	0.39

%S: % susceptible; %I: % intermediate; %R: % resistant. Green shading for %S > 80%.

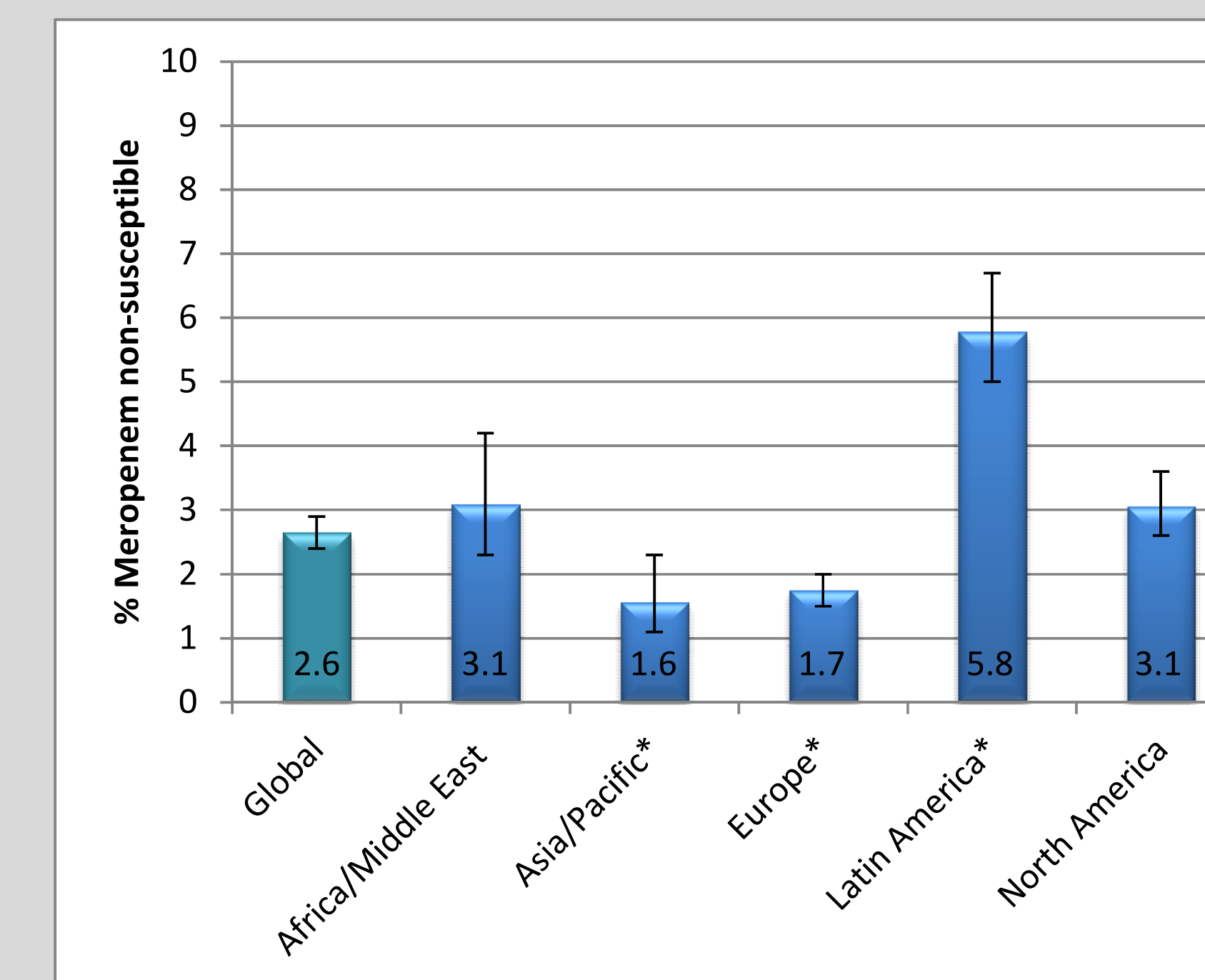
* Boldfaced p value denotes significant difference in %S between 2006/2007 and 2009/2010 ($p < 0.05$).

Table 3. Susceptibility of 592 recent meropenem non-susceptible *Enterobacteriaceae* to tigecycline and comparators by region, 2009-2010.

	% Susceptible				
	Africa/ Middle East	Asia/ Pacific	Europe	Latin America	North America
Tigecycline	86.8	81.5	90.6	79.4	82.1
Amikacin	84.2	48.2	64.9	40.0	74.5
Cefepime	47.4	25.9	32.2	16.7	57.9
Levofloxacin	57.9	40.7	30.7	22.2	37.9
Minocycline	36.8	33.3	30.2	26.7	28.3
Pip-Tazo	13.2	22.2	21.8	19.4	33.1
n	38	27	202	180	145

Green shading for % susceptible > 80%.

Figure 1. Percentage (with 95% confidence interval) of 592 recent *Enterobacteriaceae* isolates that were meropenem non-susceptible by region, 2009-10.



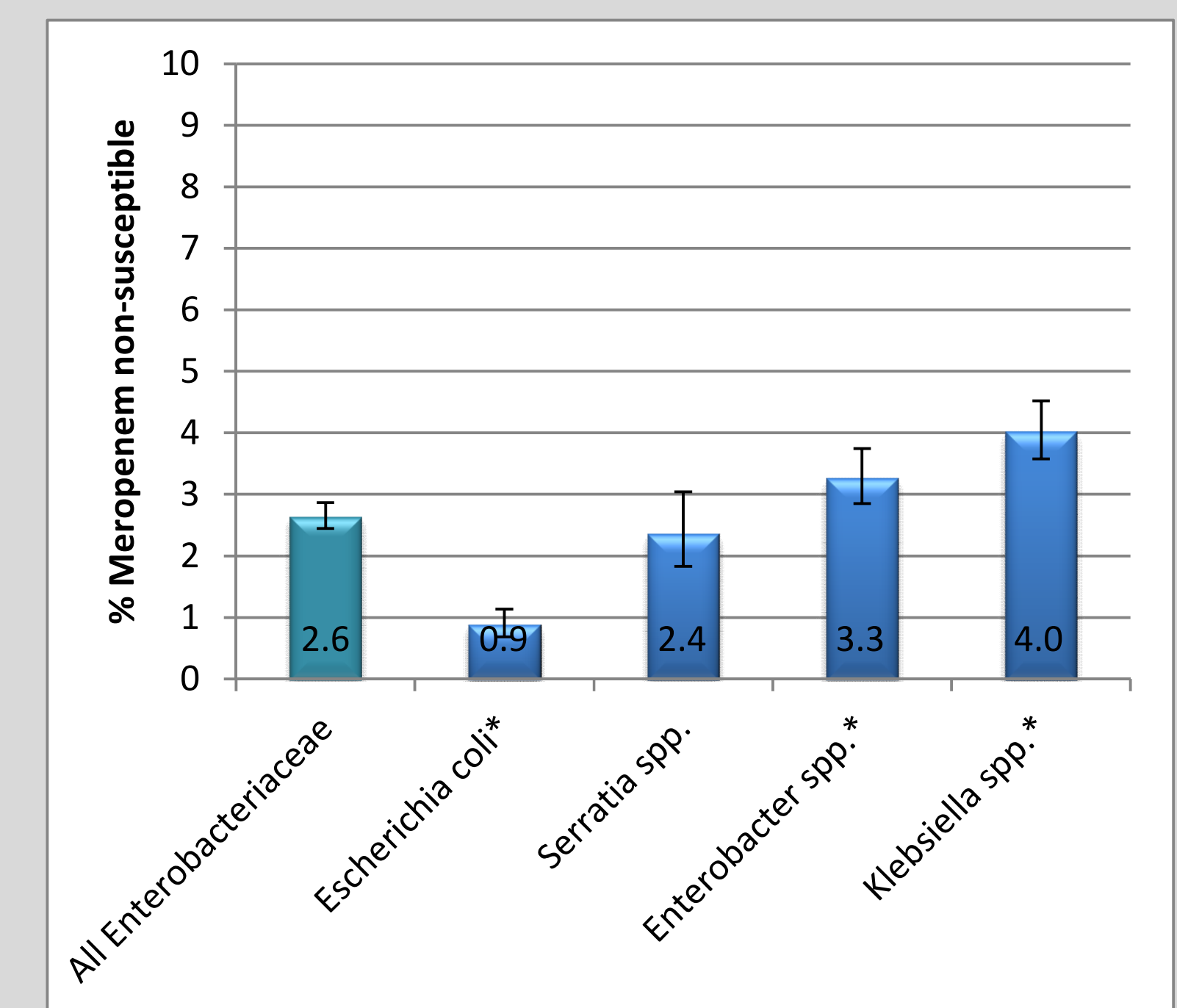
* Region is significantly different from the global average ($p < 0.05$).

Table 4. Susceptibility of 592 recent meropenem non-susceptible *Enterobacteriaceae* to tigecycline and comparators by species, 2009-10.

	% Susceptible			
	<i>Enterobacter</i> spp.	<i>Escherichia</i> <i>coli</i>	<i>Klebsiella</i> spp.	<i>Serratia</i> spp.
Tigecycline	79.2	90.3	87.7	81.4
Amikacin	68.3	50.0	54.3	69.5
Cefepime	48.5	27.4	21.2	54.2
Levofloxacin	46.0	29.0	16.4	59.3
Minocycline	23.3	32.3	32.7	30.5
Pip-Tazo	26.2	35.5	11.9	52.5
n	202	62	269	59

Green shading for % susceptible > 80%.

Figure 2. Percentage (with 95% confidence interval) of 592 recent *Enterobacteriaceae* isolates that were meropenem non-susceptible by species, 2009-2010.



* Species is significantly different from the overall proportion for all *Enterobacteriaceae* ($p < 0.05$).

Conclusions

- Although the global proportion of meropenem non-susceptible *Enterobacteriaceae* increased only slightly from 2.1% to 2.6% between 2006/2007 and 2009/2010, this increase was statistically significant ($p < 0.0001$).
- While the MIC₅₀ for tigecycline did not change between 2006/2007 and 2009/2010, the slight increase in geometric mean was statistically significant, demonstrating that this measure may offer better discrimination for changes over time. The increase in GM MIC for minocycline was also highly significant, while the decrease for cefepime was only marginally so.
- Regional rates of meropenem non-susceptible *Enterobacteriaceae* varied, with Asia/Pacific and Europe showing significantly lower rates than the global average, while in Latin America the rate was higher ($p < 0.05$). Latin America also showed lower % susceptibility for almost all reported agents than the other regions.
- The proportion of meropenem non-susceptible isolates also varied by species with significantly higher proportions in *Enterobacter* and *Klebsiella* spp., while the proportion in *E. coli* was significantly lower than the average for all species combined.
- Despite tigecycline's increase in GM MIC and a statistically significant decrease in % susceptible, the agent continued to demonstrate the best *in vitro* activity of all tested drugs against meropenem non-susceptible *Enterobacteriaceae*. Almost 85% of these usually multi-resistant pathogens remained susceptible to tigecycline globally. With the exception of amikacin in Africa/Middle East, tigecycline was also the only agent with % susceptible at about 80% or higher across all global regions as well as across species. These results confirm tigecycline as an important therapeutic option for meropenem non-susceptible *Enterobacteriaceae*, especially since the other tested antimicrobials exhibited much poorer *in vitro* activity.