

# Prevalence and Molecular Characterization of Extended Spectrum Beta-lactamase Producing *Enterobacteriaceae* in Community-Acquired Intra-abdominal Infections – SMART 2002-2009

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## Revised Abstract

**Introduction:** Extended-spectrum beta-lactamase-producing (ESBL+) *Enterobacteriaceae* often exhibit diminished susceptibility to many antibiotics in addition to the beta-lactams. These organisms, originally found primarily in hospital-acquired or healthcare-associated (HA) infections, have been increasing in prevalence worldwide over the past decade. More recently, increasing numbers of ESBL+ organisms, especially *Escherichia coli*, have been reported in community-acquired (CA) infections. The Study for Monitoring Antimicrobial Resistance Trends (SMART) has monitored global and regional resistance rates among pathogens causing HA and CA intra-abdominal infections (IAI) since 2002. This report describes increasing prevalence of ESBL+ isolates in CA IAI from 2002-2009, and summarizes their susceptibility to drugs commonly used to treat IAI.

**Methods:** 257 hospitals in 44 countries collected 16,214 isolates of *E. coli*, *Klebsiella pneumoniae*, *K. oxytoca*, and *Proteus mirabilis* from patients with CA IAI from 2002-2009. Susceptibility and ESBL testing were done using broth microdilution based on CLSI methods, and interpreted using CLSI 2010 (January, M100-S20) standards. All ESBL+ or carbapenem-resistant isolates from 2008-2009 were molecularly characterized at a central lab (IHMA, Inc.) using PCR sequencing.

**Results:** Worldwide, ESBL+ CA IAI isolates increased from 5% in 2002 to 14% in 2009, with the largest increases observed in Asia and Latin America. 90% of the 2008-2009 isolates possessed CTX-M enzymes (61% of which were CTX-M15, 20% CTX-M14, and 19% were of 15 other variants); 10% had SHVs; 2% TEMs; and <1% KPC. Susceptibility of ESBL+ isolates was >70% to only 5 drugs: imipenem (99%), ertapenem (98%), amikacin (87%), pip-tazo (79%), and cefoxitin (76%). Susceptibility to ceftazidime, levofloxacin, ciprofloxacin, cefepime, amp-sulbactam, ceftriaxone, and cefotaxime were all <30%.

**Conclusions:** ESBL+ rates in CA IAI have increased over 280% since 2002, mostly due to increases in Asia and Latin America. CTX-M-15 and CTX-M-14 enzymes are by far the most common ESBLs found in current CA IAI, while the KPC rate was extremely low. In areas where ESBL+ rates in CA IAI are high, effective empiric therapy may be limited to carbapenems, amikacin, pip-tazo, or the few other agents shown to have activity against ESBL+ isolates.

## Introduction

The emergence of antimicrobial resistance in bacteria is a global healthcare problem. Particularly troubling has been the spread of extended spectrum beta-lactamase producing (ESBL+) gram-negative pathogens that have rendered all but a few antibiotics ineffective and is associated with increased hospital stay, costs, and mortality [1,2]. Several collaborative studies have been developed to monitor resistance [3-5]. The Study for Monitoring Antimicrobial Resistance Trends (SMART) was begun in 2002 and is the only surveillance study to globally monitor the *in vitro* antimicrobial activity in both hospital- and community-associated gram-negative aerobic and facultative intra-abdominal infections (IAI). This report from the SMART data describes the increasing prevalence of ESBL+ isolates in community-associated IAI from 2002-2009, their molecular characterizations (2008-2009 isolates only), and summarizes their susceptibility to drugs commonly used to treat IAI.

## Materials & Methods

- Demographics:** 257 hospitals in 44 countries collected 16,214 isolates of *E. coli*, *Klebsiella pneumoniae*, *K. oxytoca*, and *Proteus mirabilis*. All were non-repeat isolates derived from community-associated IAIs from 2002 to 2009.
- Antimicrobial susceptibility testing** and interpretive criteria followed published guidelines of the Clinical and Laboratory Standards Institute (CLSI). Minimum inhibitory concentrations (MICs) were determined using MicroScan dehydrated broth microdilution panels (Siemens Medical Solutions Diagnostics, West Sacramento, California, USA) following CLSI guidelines [6].
- ESBL phenotypic tests** for *E. coli*, *K. pneumoniae*, *K. oxytoca* and *P. mirabilis* isolates were classified as positive according to CLSI guidelines when there was at least an eight-fold reduction of an MIC for ceftazidime or cefotaxime tested in combination with clavulanic acid compared to the MIC of the antibiotic when tested without clavulanic acid [7].
- Molecular characterizations** were performed on whole DNA extracts obtained using the QIAgen DNA Mini kit and the QIAcube instrument (Qiagen, Valencia, CA). These DNA extracts were used for the Check-Points (Check-Points, Wageningen, The Netherlands) and PCR sequencing. Identification of *bla*<sub>ESBL</sub> and *bla*<sub>KPC</sub> genes was determined using the Check-KPC ESBL system. PCR was performed using specific primers designed for identifying β-lactamase genes including *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub> and *bla*<sub>CTX-M</sub> from groups 1, 2, 9 and 8/25 according to the Check-Points results. The presence of plasmid-mediated AmpC genes in all isolates was determined by multiplex-PCR. Sequencing was performed using the same specific primers as PCR.
- Quality control** testing (QC) was done on each day of testing using the CLSI-recommended QC strains: *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603 (ESBL+ control), and *Pseudomonas aeruginosa* ATCC 27853. Results were included in the analysis only when corresponding QC isolates tested within the acceptable range according to CLSI guidelines [7].
- A centralized database** of the study results was developed and managed by International Health Management Associates, Inc. (IHMA, Schaumburg, IL, USA).

## References

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## Acknowledgements

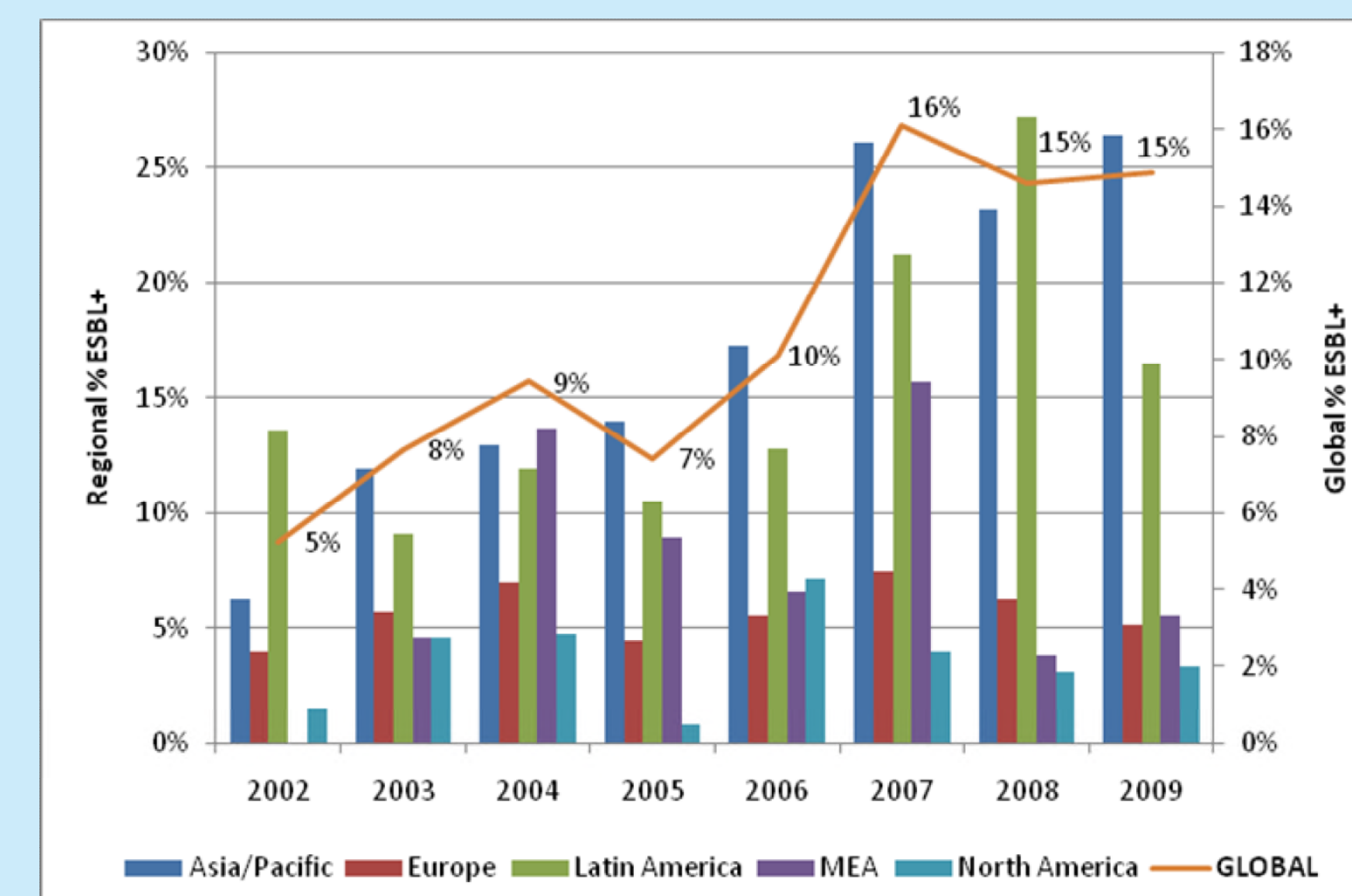
The authors thank all the participants in the SMART program, the success of which relies upon their ongoing support and input. The SMART program is funded by Merck Research Laboratories.

Table 1. Total Number and Percentage of Community-Associated ESBL+ Isolates from 2002 - 2009.

Organisms	Total No. of Isolates	No. of ESBL+ Isolates	% ESBL+
<i>E. coli</i> *	12,001	1362	11.4
<i>K. pneumoniae</i> *	2,818	376	13.3
<i>K. oxytoca</i>	680	42	6.2
<i>P. mirabilis</i>	715	41	5.7
<b>Grand Totals</b>	<b>16,214</b>	<b>1,821</b>	<b>11.2</b>

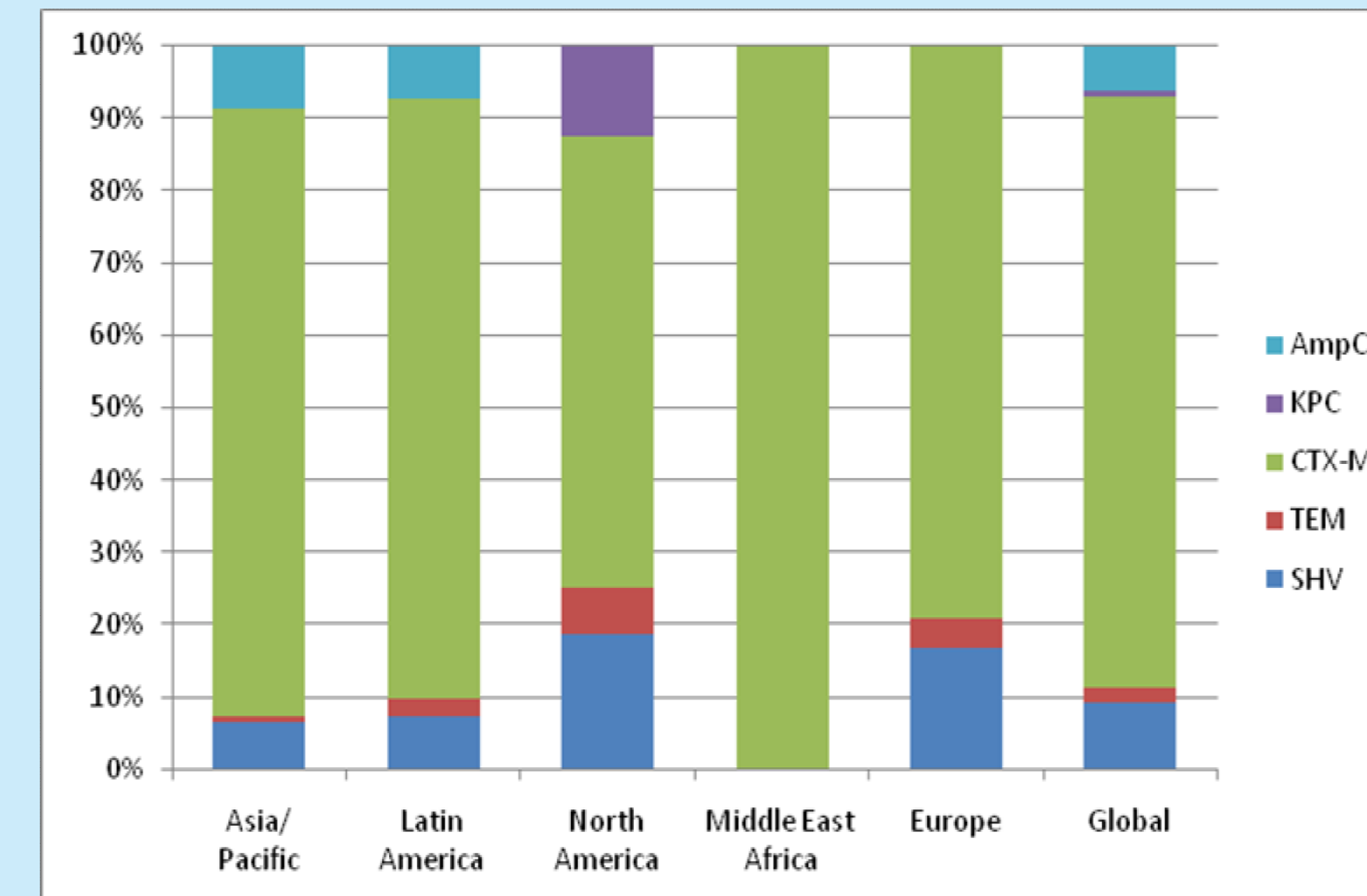
\* Only *E. coli* and *K. pneumoniae* had sufficient numbers of ESBL+ isolates to analyze by year.

Figure 2. Community-Associated ESBL+ *E. coli* Rates in IAI - SMART 2002-2009, Global and Region.



P-value <0.001 for global data, 2002 compared to 2009 (Fisher's Exact Test, two-tailed).

Figure 4. Relative Percentage of Beta-lactamase Types Found in Community-Associated Carbapenem-Resistant or ESBL+ *Enterobacteriaceae* from 2008 - 2009\* by Region (n=259).



\* Molecular characterizations were begun in 2008. Isolates prior to 2008 are not available for testing.

## Results

Figure 1. Percent Susceptible of Community-Associated ESBL+ *Enterobacteriaceae*\* from 2002-2009 (n=1,821).

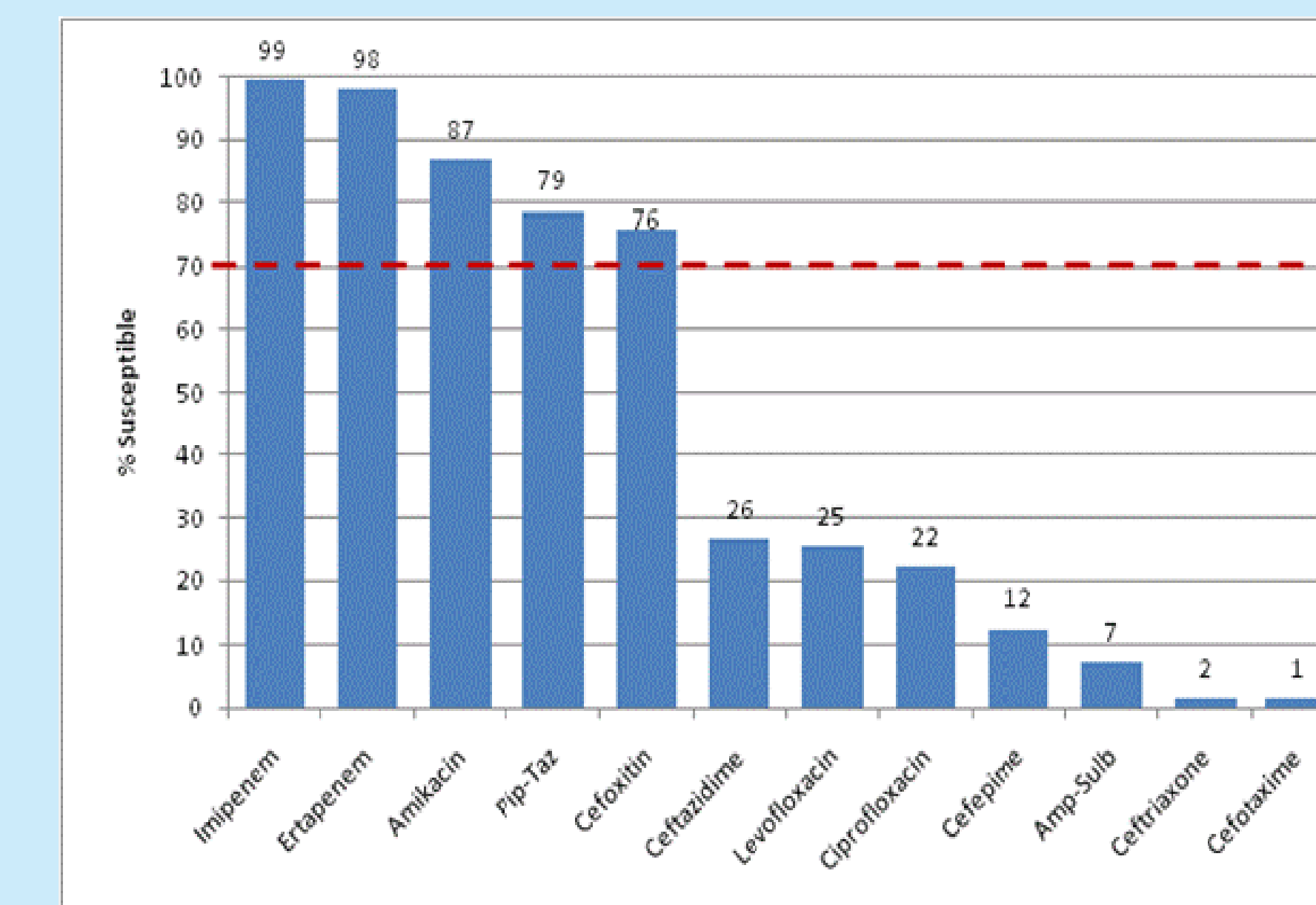
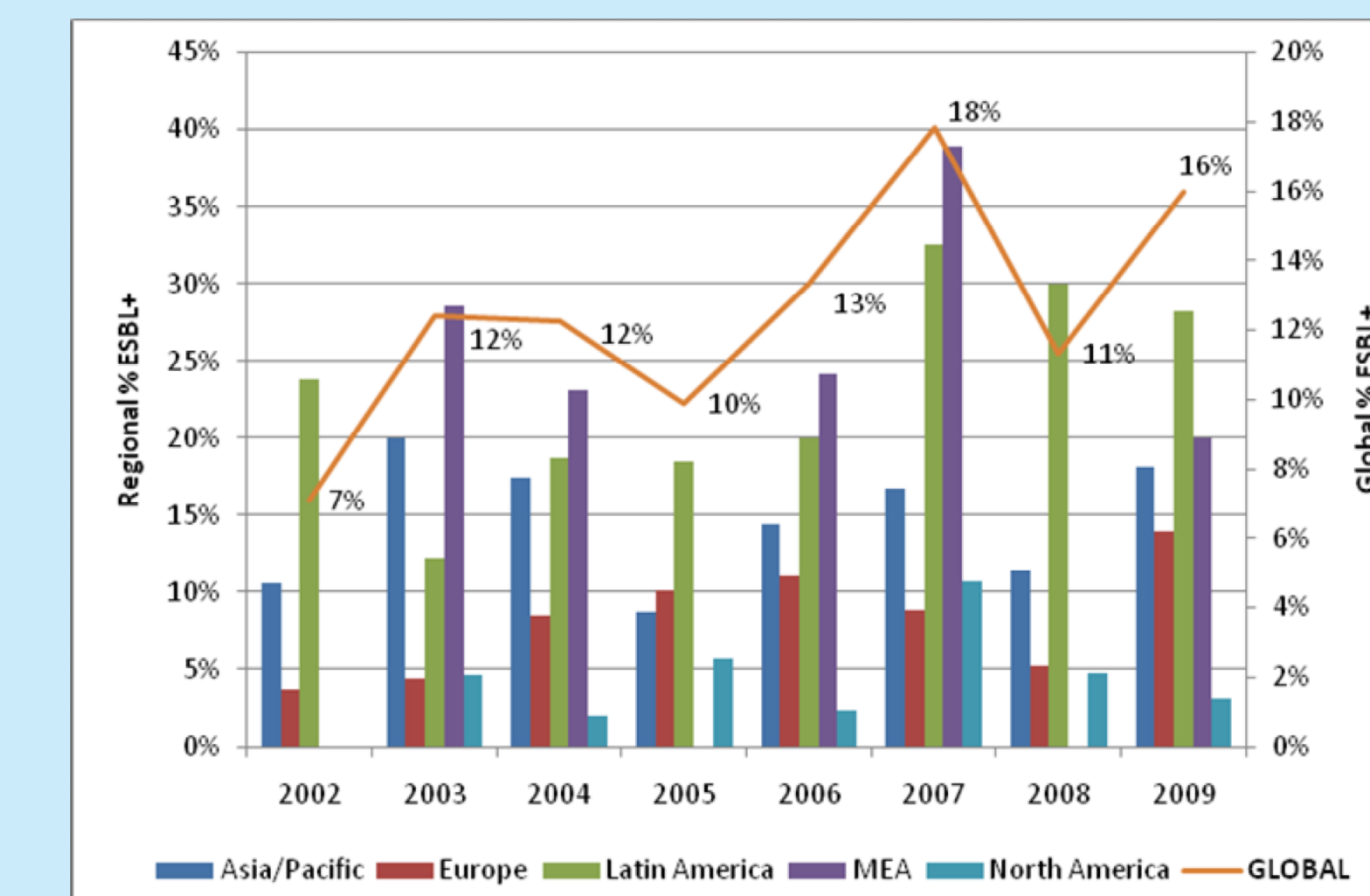
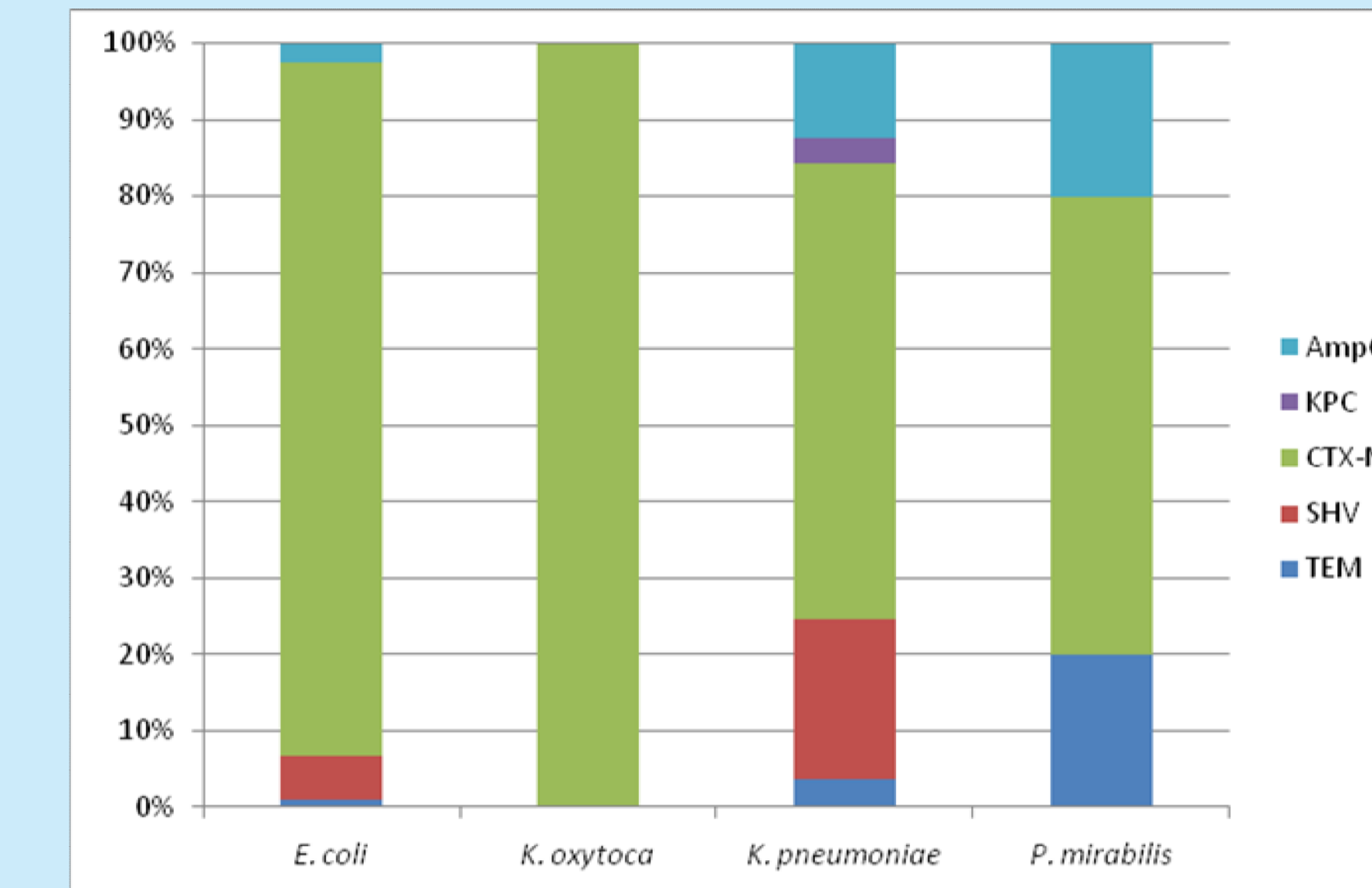


Figure 3. Community-Associated ESBL+ *K. pneumoniae* Rates in IAI - SMART 2002-2009, Global and Region.



P-value <0.01 for global data, 2002 compared to 2009 (Fisher's Exact Test, two-tailed).

Figure 5. Relative Percentage of Beta-Lactamase Types Found in Community-Associated Carbapenem-Resistant or ESBL+ *Enterobacteriaceae* from 2008 - 2009\* by Species (n=259).



\* Molecular characterizations were begun in 2008. Isolates prior to 2008 are not available for testing.

Table 2. Percent Susceptible (%) of Community-Associated ESBL+ *Enterobacteriaceae* by Genotype\*, 2008-2009 (n=259).

Drug	CTX-M** (n=232)	CTX-M14 (n=47)	CTX-M15 (n=142)	KPC (n=2)	SHV (n=26)	TEM (n=6)	Total (n=259)
Imipenem	100	100	100	50	100	100	99
Ertapenem	100	98	100	0	96	100	99
Pip-Tazo	79	89	75	0	50	100	76
Cefoxitin	75	70	77	0	65	67	73
Amikacin	66	83	57	0	65	67	66
Ceftazidime	29	75	6	0	12	17	25
Levofloxacin	22	34	17	0	50	83	25
Ciprofloxacin	19	32	12	0	42	67	21
Cefepime	5	4	5	0	50	17	9
Amp-Sulb	4	0	3	0	15	33	6
Ceftriaxone	0	0	0	0	0	0	0
Cefotaxime	0	0	0	0	0	0	0

\* Molecular characterization was started in 2008. Isolates prior to this date are unavailable for testing.  
 \*\* CTX-M includes the CTX-M14, CTX-M15 genotypes presented in this table and 15 other minor variants not represented.

## Conclusions

- There has been a significant rise from 2002 to 2009 in global ESBL rates in community-associated IAI, from 5% to 15% for *E. coli* (p <0.001) and 7% to 16% for *K. pneumoniae* (p <0.01). The largest increases were observed primarily in the Asia/Pacific and Latin America regions.
- CTX-M enzymes, especially CTX-M-14 and CTX-M-15, were the predominant ESBL genotype observed in IAI infection from 2008 - 2009, accounting for 90% of all ESBLs detected. SHV, CTX-M, TEM, and AmpC enzymes had considerable variation among the study species.
- KPC enzymes are frequently reported in current literature for hospital-associated isolates but were rare (n=2) in the community-associated isolates of this study. The two KPC-producing isolates were resistant to all drugs in the study except imipenem using M100-S20 CLSI breakpoints (Jan 2010); however, the imipenem MIC of 2 mcg/ml for one of the isolates is intermediate using the new CLSI M100-S20-U breakpoints (July 2010).
- Only imipenem, ertapenem, and amikacin maintained *in vitro* activity of >85% against global community-associated ESBL+ isolates from 2002 - 2009. Piperacillin-tazobactam and cefoxitin were close behind with susceptibility rates of 79% and 76%, respectively. The remaining study drugs all had susceptibilities <30%. In areas where ESBL+ rates in CA IAI are high, effective empiric therapy may be limited to carbapenems, amikacin, piperacillin-tazobactam, or the few other agents shown to have activity against ESBL+ isolates.