

Revised Abstract

Background: In recent years carbapenem resistance has emerged among gram-negative isolates due to the acquisition of carbapenemases, which usually belong to Ambler class B metallo-beta-lactamases (MBLs) or to KPC-type enzymes. *Klebsiella pneumoniae* carbapenemase (KPC) is an Ambler class A beta-lactamase that confers resistance to all beta-lactam agents, including carbapenems, cephalosporins, penicillins, and the monobactam aztreonam. Although this enzyme has been found primarily in *K. pneumoniae*, it has also been identified in several other gram-negative bacilli. Because the *bla*_{KPC} gene is carried on a plasmid, the ease of mobility of this resistance mechanism is of concern and represents a major threat to the antimicrobial treatment of infections with gram-negative organisms. As part of the Tigecycline European Surveillance Trial (TEST), this study investigated the incidence and susceptibility profiles of *bla*_{KPC} positive gram-negative isolates from Europe during 2009-10. **Methods:** 11,316 *Enterobacteriaceae* isolated in Europe in 2009-10 had minimum inhibitory concentrations (MICs) determined using broth microdilution following CLSI guidelines and interpreted according to EUCAST breakpoints where available. A total of 148 isolates with meropenem MICs of ≥ 2 mg/L were screened for the presence of KPC genes using multiplex-PCR.

Results: Of 11,316 *Enterobacteriaceae* isolated in Europe in 2009-10, 148 (1.3%) had meropenem MICs of ≥ 2 mg/L. 52 (35%) of the 148 meropenem non-susceptible isolates were positive for KPC genes. All *bla*_{KPC} positive isolates were *K. pneumoniae*. Susceptibility of these isolates is shown below:

	MIC ₅₀	MIC ₉₀	%S	%I	%R
Tigecycline	1	1	94.2	1.9	3.9
Minocycline*	4	8	63.5	28.8	7.7
Amikacin	16	>64	19.2	40.4	40.4
Levofloxacin	>8	>8	7.7	1.2	91.1
Cefepime	>32	>32	0	0	100
Ceftriaxone	>64	>64	0	0	100
Meropenem	>16	>16	0	0	100
Pip-Tazo	>128	>128	0	0	100

MIC₅₀ in mg/L; *Minocycline breakpoints based on CLSI (2011)

Conclusions: The meropenem non-susceptible rate in European *Enterobacteriaceae* from the TEST study in 2009-10 was 1.3%, with 35% of these (0.5% of total *Enterobacteriaceae*) positive for *bla*_{KPC}. The most active antimicrobial against these isolates *in vitro* was tigecycline with 94.2% of isolates susceptible, followed by minocycline, with 63.5% susceptible. All other antimicrobials exhibited susceptibilities of <20%.

Introduction

Carbapenems are commonly used to treat infections caused by *Enterobacteriaceae*. In recent years, carbapenem resistance has emerged among gram-negative isolates due to the acquisition of carbapenemases, which usually belong to Ambler class B metallo-beta-lactamases (MBLs) or to KPC-type enzymes. *Klebsiella pneumoniae* carbapenemase (KPC) is an Ambler class A beta-lactamase that confers resistance to all beta-lactam agents, including carbapenems, cephalosporins, penicillins, and the monobactam aztreonam. Although this enzyme has been found primarily in *K. pneumoniae*, it has also been identified in several other gram-negative bacilli. Because the *bla*_{KPC} gene is carried on a plasmid, the ease of mobility of this resistance mechanism is of concern and represents a major threat to the antimicrobial treatment of infections with gram-negative organisms. The Tigecycline European Surveillance Trial (TEST) has been monitoring antibiotic susceptibilities since 2004. In this study, we investigated the incidence and susceptibility profiles of *bla*_{KPC} positive gram-negative isolates from Europe during 2009-10.

Materials & Methods

➤ All TEST isolates were derived from blood, respiratory tract, urine (no more than 25% of all isolates), skin, wound, fluids and few other defined sources. Isolates were identified to genus and species at each site by the local laboratory. MICs were determined on each isolate by the local laboratory. Only one isolate per patient was accepted.

➤ Clinical isolates for this analysis were collected and tested between January 2009 and December 2010 from 12 countries in Europe (Austria, Bulgaria, Croatia, France, Germany, Greece, Italy, Poland, Portugal, Romania, Spain and Turkey).

➤ Custom broth microdilution panels were supplied by TREK (TREK Diagnostic Systems, Cleveland, OH) with the following antimicrobial agents and concentrations (expressed in mg/L): amoxicillin/clavulanic acid (0.12/0.06-32/16); piperacillin/tazobactam (0.06-128); levofloxacin (0.008-8); ceftriaxone (0.06-64); cefepime (0.5-32); ampicillin (0.5-32); amikacin (0.5-64); minocycline (0.5-16); ceftazidime (8-32); tigecycline (0.008-16); and meropenem (0.12-16).

➤ Minimum inhibitory concentrations (MICs) were determined by the CLSI recommended broth microdilution testing method [1]. MIC interpretive criteria followed published guidelines established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [2] where applicable; minocycline breakpoints defined by CLSI [3]; tigecycline breakpoints defined by the FDA [4].

➤ Quality control of broth microdilution panels followed manufacturer and CLSI guidelines using *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922.

➤ DNA extraction was carried out as follows: A suspension of overnight colonies was heated for 5 min at 95°C and immediately frozen at -20°C for at least 5 min in 96 wells-plates. DNA was extracted using the QIAamp DNA Mini Kit and the QIAcube instrument (Qiagen, Valencia, CA). Further characterization of isolates via multiplex PCR utilized the Check-Points system (Check MDR CT 101, Check-Points, The Netherlands). The following primers were used for *bla*_{KPC} amplification [5]:

KPCF	TGCTACTGTATCGCCGTC
KPCR	CTCAGTGTCTACAGAAAACC

References

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5. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, Alberti S, Bush K, Tenover FC. 2001. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. 45: 1151-61.

Acknowledgements

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Results

Table 1. *In vitro* activity of eight antimicrobial agents against 11,316 *Enterobacteriaceae* from Europe, 2009-2010.

	MIC ₅₀	MIC ₉₀	%S*	%I	%R
Tigecycline	0.5	2	89.4	7.7	2.9
Minocycline	4	16	64.0	17.8	18.2
Amikacin	2	8	94.5	2.8	2.7
Levofloxacin	0.12	>8	75.7	2.5	21.7
Cefepime	≤ 0.5	32	76.2	8.2	15.6
Ceftriaxone	0.12	>64	68.7	1.9	29.4
Meropenem	≤ 0.06	0.12	98.9	0.7	0.3
Pip-Tazo	2	128	74.6	5.4	20.0

*MIC expressed in mg/L; breakpoint interpretations based on EUCAST guidelines [2] except tigecycline, which is based on FDA guidelines [4] and minocycline, which is based on CLSI [3]

Table 2. *In vitro* activity of eight antimicrobial agents against 52 *K. pneumoniae* positive for *bla*_{KPC} from Europe, 2009-2010.

	MIC ₅₀	MIC ₉₀	%S*	%I	%R
Tigecycline	1	1	94.2	1.9	3.9
Minocycline	4	8	63.5	28.8	7.7
Amikacin	16	>64	19.2	40.4	40.4
Levofloxacin	>8	>8	7.7	1.2	91.1
Cefepime	>32	>32	0	0	100
Ceftriaxone	>64	>64	0	0	100
Meropenem	>16	>16	0	0	100
Pip-Tazo	>128	>128	0	0	100

*MIC expressed in mg/L; breakpoint interpretations based on EUCAST guidelines [2] except tigecycline, which is based on FDA guidelines [4] and minocycline, which is based on CLSI [3]

Figure 1. Distribution of 52 *K. pneumoniae* positive for *bla*_{KPC} from Europe by specimen type.

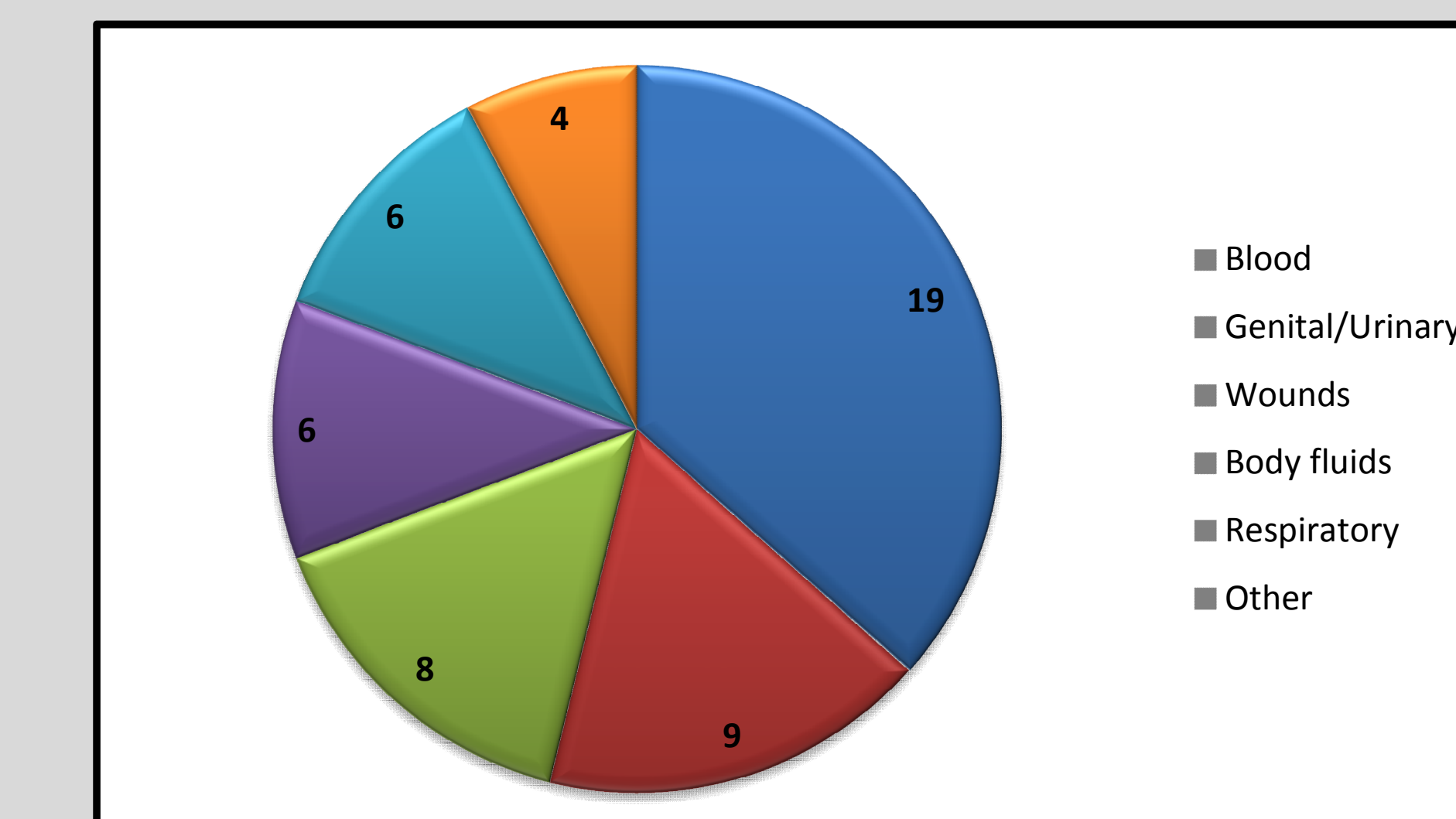


Figure 2. Distribution of 52 *K. pneumoniae* positive for *bla*_{KPC} from Europe by hospital unit.

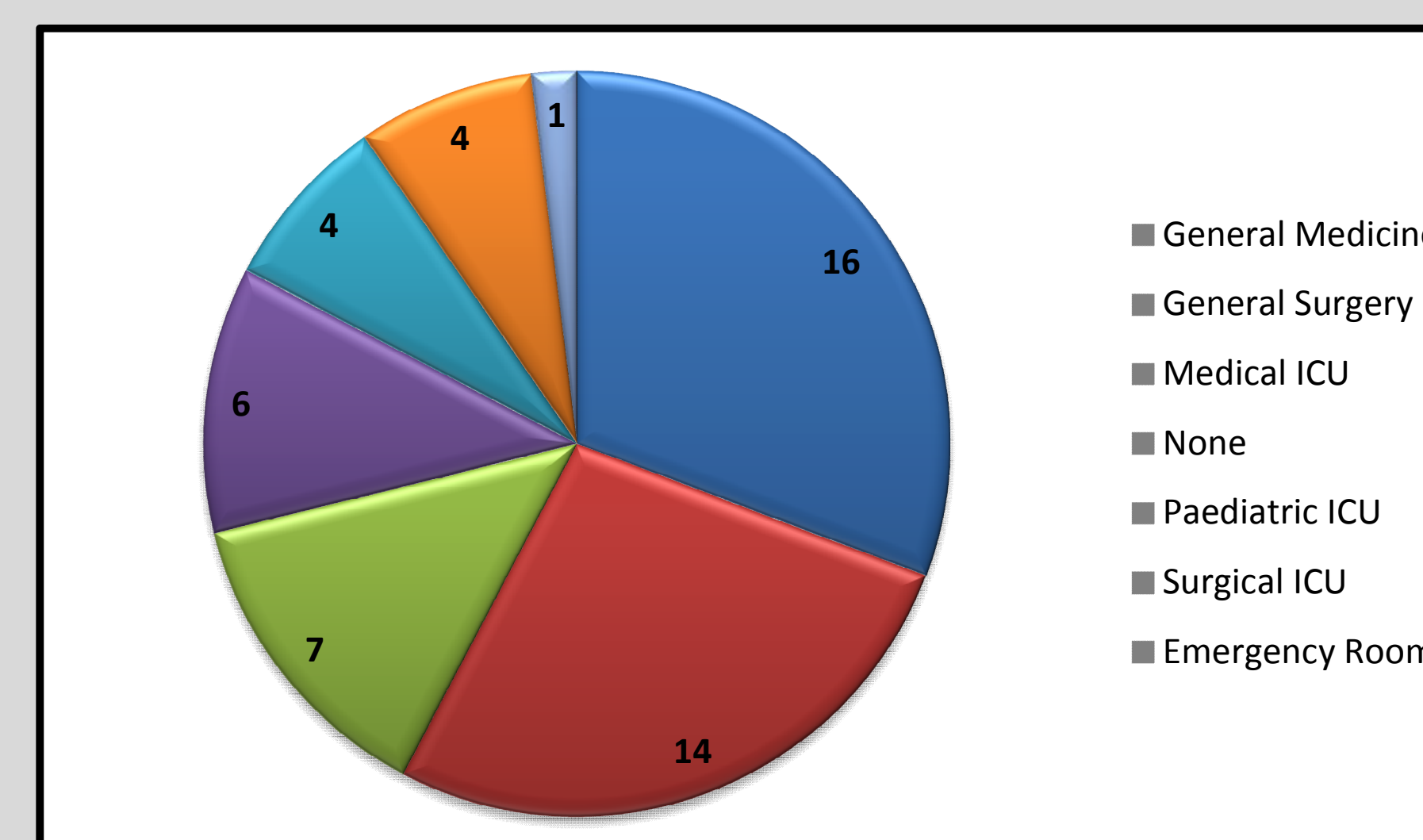


Figure 3. Total number of *K. pneumoniae* (KPN), KPN with meropenem (MER) MIC ≥ 2 mg/L and KPN producing KPC by country.

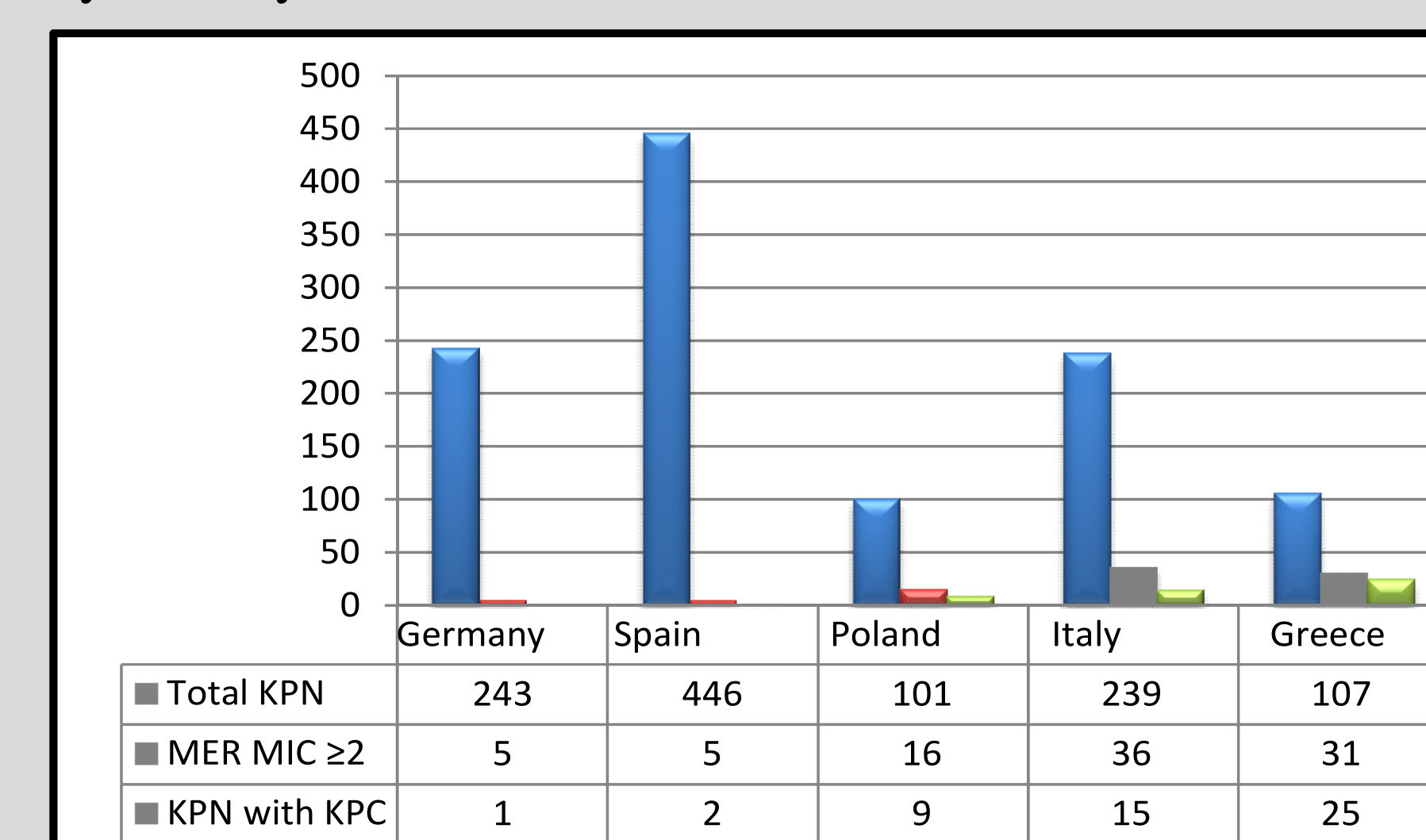
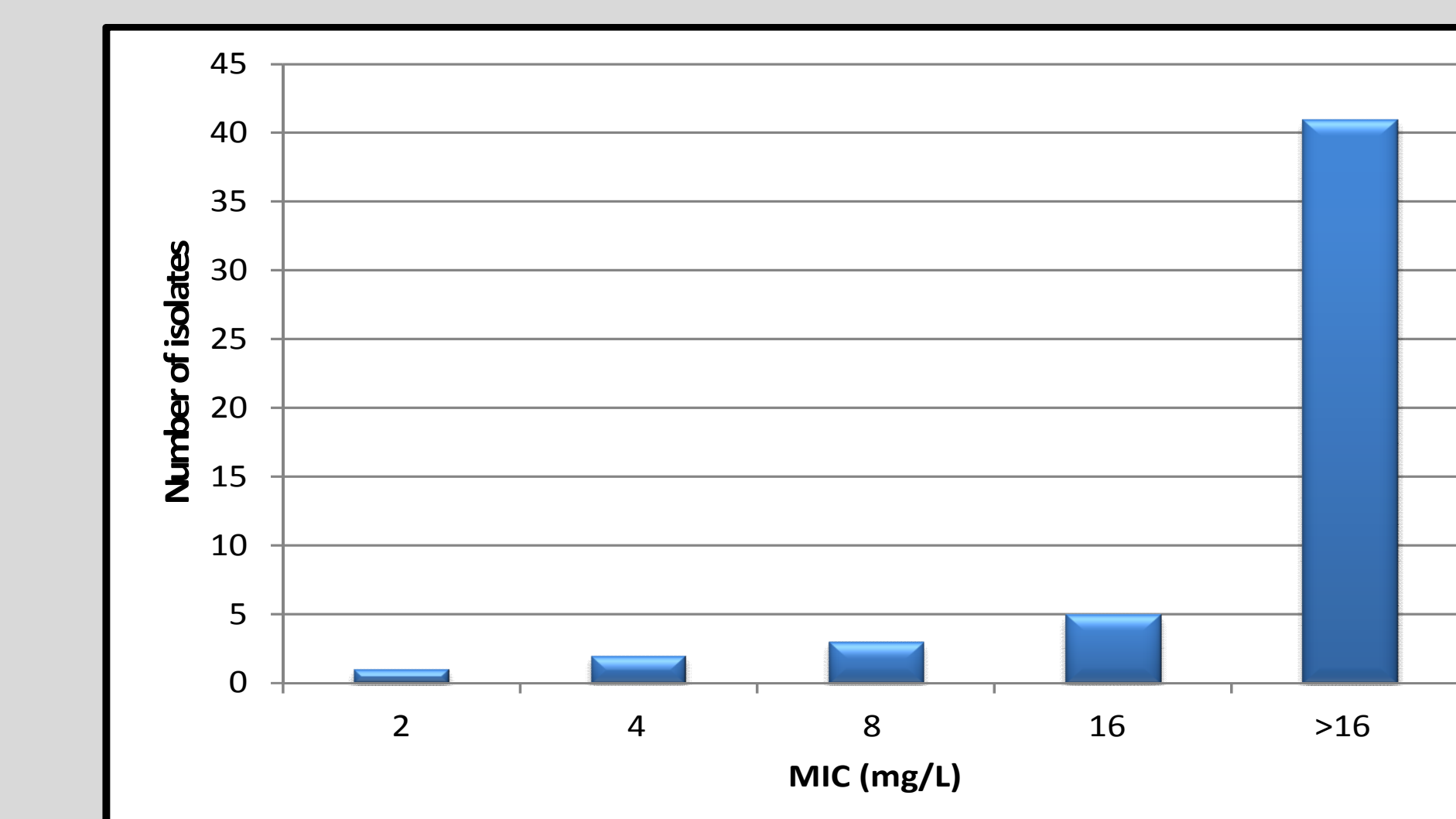


Figure 4. Meropenem MIC distribution of 52 *K. pneumoniae* positive for *bla*_{KPC} from Europe.



Conclusions

- The meropenem non-susceptible rate in European *Enterobacteriaceae* from the TEST study in 2009-10 was 1.3%, with 35% of these (0.5% of total *Enterobacteriaceae*) positive for *bla*_{KPC}.
- The most active antimicrobial against these *bla*_{KPC} isolates *in vitro* was tigecycline with 94.2% of isolates susceptible, followed by minocycline, with 63.5% susceptible. All other antimicrobials exhibited susceptibilities of <20%.
- *bla*_{KPC} was most common in *K. pneumoniae* from Greece (23.4%), followed by Poland (8.9%) and Italy (6.2%).