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SUPPLEMENT ARTICLE

Variations in the Occurrence of Resistance Phenotypes and Carbapenemase Genes Among *Enterobacteriaceae* Isolates in 20 Years of the SENTRY Antimicrobial Surveillance Program

Mariana Castanheira,[1] Lalitagauri M. Deshpande,[1] Rodrigo E. Mendes,[1] Rafael Canton,[2] Helio S. Sader,[1] and Ronald N. Jones[1]

[1]JMI Laboratories, North Liberty, Iowa; [2]Servicio de Microbiología, Hospital Universitario Ramón y Cajal and Instituto Ramón y Cajal de Investigación Sanitaria, Madrid, Spain

Background. A total of 178 825 *Enterobacteriaceae* isolates collected in 199 hospitals from 42 countries worldwide over 20 years (1997 to 2016) of the SENTRY Program were susceptibility tested by reference broth microdilution methods.

Methods. Trends in percentages over time were analyzed by the χ^2 test. Results were reported as the percentage difference between the first (1997–2000) and the last (2013–2016) time period.

Results. *Enterobacteriaceae* exhibiting resistance to cephalosporins (extended-spectrum β-lactamase [ESBL] phenotype) and carbapenem resistance (CRE) significantly increased ($P < 0.05$; χ^2 test) from 10.3% to 24.0% and 0.6% to 2.9%, respectively. Similar trends were noted for all regions and infection sources. *Klebsiella pneumoniae* mainly drove the CRE increase. Multidrug-resistance (MDR) rates significantly increased from 7.3% to 15.3% overall, with important trends in all regions and infection sources. Significant increases were noted for MDR *K. pneumoniae* and *Escherichia coli*, polymyxin-resistant *K. pneumoniae* (2.0% to 5.5% overall), and aminoglycoside-resistant *E. coli* (7.0% to 18.0%) and *K. pneumoniae* (18.1% to 26.9%) over time in North America and Latin America. Carbapenemase-encoding genes were screened after 2007, and the occurrence of these genes was compared for 2007–2009 and 2014–2016. Among 1298 CRE isolates from the 2 study periods, bla_{KPC} was detected among 186 (49.7%) and 501 (54.2%) isolates in 2007–2009 and 2014–2016, respectively. Metallo-β-lactamase genes were detected among 4.3% of the isolates from 2007 to 2009 and 12.7% of the isolates from 2014 to 2016, mainly due to the dissemination of isolates carrying bla_{NDM}. Genes encoding IMP and VIM enzymes were observed in 1.9% and 2.4% (7 and 9 isolates) of the isolates from 2007 to 2009 and 0.4% and 1.9% of the isolates from 2014 to 2016. OXA-48 and variants increased from 4.3% in 2007–2009 to 12.6% in 2014–2016 (mainly in Europe).

Conclusions. A change in the epidemiology of carbapenemases and important increases in ESBL, CRE, MDR, and other resistant phenotypes among virtually all geographic regions and infection sources were noted in the 20 years of surveillance, highlighting the impact of antimicrobial resistance and the importance of its continuous monitoring.

Keywords. carbapenemases; CRE; *Enterobacteriaceae*; ESBL; MDR.

Acquired resistance among species belonging to the *Enterobacteriaceae* family limits the antimicrobial therapeutic options for infections caused by these organisms and is a growing cause of concern. Among the numerous resistance mechanisms observed in *Enterobacteriaceae* species, β-lactamases are especially worrisome because they limit the use of β-lactam agents that have broad spectrum of activity and excellent safety profiles [1]. *Enterobacteriaceae* isolates producing extended-spectrum β-lactamases (ESBLs), plasmid-encoded cephalosporinases, or carbapenemases are resistant to some or most β-lactams that are used as the traditional first-line options for the treatment of serious infections caused by these pathogens [2].

In addition, *Enterobacteriaceae* isolates producing β-lactamases often coharbor resistance mechanisms against other antimicrobial classes. Genes encoding resistance to fluoroquinolones, aminoglycosides, tetracyclines, and trimethoprim-sulfamethoxazole are often carried by mobile genetic elements that also carry β-lactamases, promoting the dissemination of resistance to multiple antimicrobial agents concomitantly [3]. Moreover, mutation-driven resistance mechanisms that reduce the affinity of the bacterial target to the antimicrobial agent or cause changes in the expression of outer membrane proteins (porins) or efflux pump systems contribute to a multidrug-resistant (MDR) phenotype among species of the *Enterobacteriaceae* family.

Multidrug-resistant *Enterobacteriaceae* isolates that were once uncommon have been reported with increasing frequency. In the

Correspondence: M. Castanheira, PhD, JMI Laboratories, 345 Beaver Kreek Centre, Suite A, North Liberty, IA 52317 (mariana-castanheira@jmilabs.com).

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European Antimicrobial Resistance Surveillance Network, resistance among *Escherichia coli* isolates to 3 antimicrobial classes that included the fluoroquinolones, cephalosporins, and aminoglycosides and would be considered MDR ranged from approximately 1% in 2002 [4] to 4.8% in 2016 [5]. In this European survey, MDR *Klebsiella pneumoniae* rates decreased from 18.9% in 2013 to 15.8% in 2016; however, in 16 European countries these rates ranged from 16.9% to 55.7% in 2016 [5].

Studies demonstrate that inappropriate antimicrobial therapy associated with β-lactamase production and MDR in *Enterobacteriaceae* species causes higher morbidity and mortality, significantly higher hospital costs, and prolonged hospital stays [3, 6]. Surveillance of antimicrobial resistance is recognized as an important tool at the local, national, and global levels for providing information to (1) establish better guidelines for empiric antimicrobial therapy, (2) promote awareness, and (3) prevent the dissemination of antimicrobial resistance. The SENTRY Antimicrobial Surveillance Program was initiated in 1997, and for over 20 years it has collected and published data on the global and regional resistance levels of the main organisms causing important bacterial and fungal infections.

In this study, we analyzed the trends of resistance phenotypes in the main antimicrobial classes and carbapenemase production among 178 825 *Enterobacteriaceae* isolates collected in 199 hospitals from 42 countries over 20 years (1997–2016) of the SENTRY Antimicrobial Surveillance Program.

METHODS

Bacterial Isolates

A total of 178 825 *Enterobacteriaceae* isolates were collected during 1997–2016 from 199 medical centers participating in the SENTRY Program that were distributed in 42 countries located in 4 main geographic regions (Figure 1A). Each participating hospital was asked to submit 1 isolate per patient episode of consecutive bacterial isolates from bloodstream infections (BSIs), skin and skin structure infections (SSSIs), pneumonia in hospitalized patients, urinary tract infections (UTIs), and intra-abdominal tract infections (Figure 1B) determined to be significant by local criteria as the reported cause of infection. Bacterial identification was primarily performed at the participating hospital and confirmed, as needed, using biochemical methods (1997–2011) and/or matrix-assisted laser desorption ionization-time of flight mass spectrometry (2012–2016).

Susceptibility Testing

Organisms were susceptibility tested by reference broth microdilution methods in a central laboratory according to the current Clinical and Laboratory Standards Institute (CLSI) documents [7, 8]. Validated broth microdilution panels were manufactured at JMI Laboratories (2015–2016) or by Thermo Fisher Scientific (Cleveland, OH) (1997–2014). Quality control (QC) was performed according to CLSI guidelines, and all QC minimum

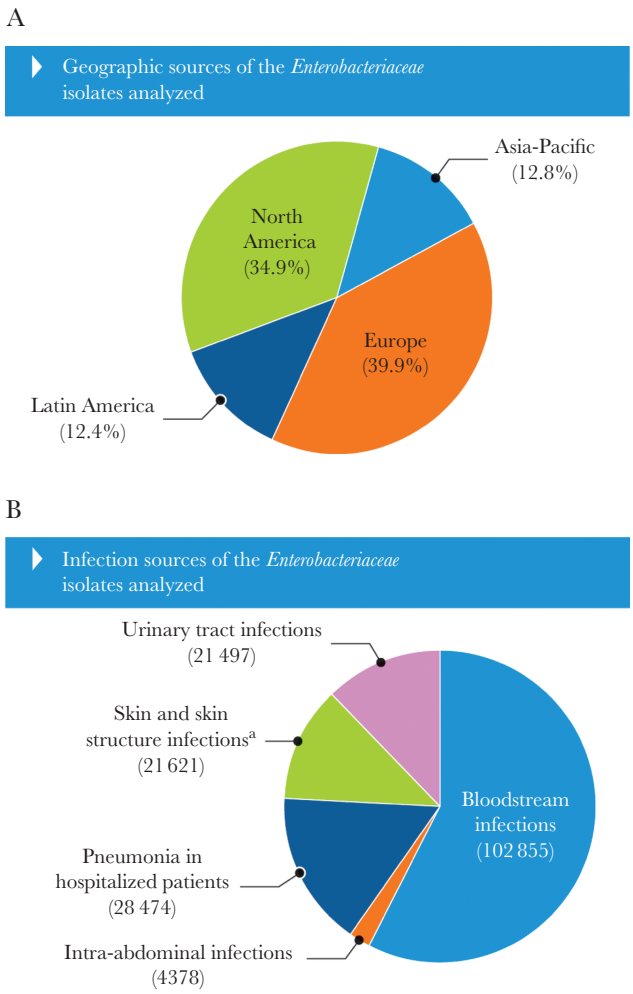


Figure 1. Geographic (A) and infection sources (B) of the *Enterobacteriaceae* isolates analyzed. [a]Skin and skin structure infection isolates were mainly recovered from wounds and abscesses.

inhibitory concentration (MIC) results were within acceptable ranges as published in CLSI documents [7]. Categorical interpretations for antimicrobial agents were those found in the CLSI document M100 Ed28 [7], European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint tables [9], and/or the US Food and Drug Administration website (https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/ucm410971.htm).

Phenotype Definitions

The CLSI ESBL-phenotype criteria for epidemiological detection of ESBL-producing organisms was used for *E. coli*, *K. pneumoniae*, *Klebsiella oxytoca*, and *Proteus mirabilis* and was defined as an MIC value ≥2 mg/L for ceftriaxone, ceftazidime, and/or aztreonam [7].

Isolates exhibiting doripenem, imipenem, and/or meropenem MIC values at ≥2 mg/L were categorized as carbapenem-resistant *Enterobacteriaceae* (CRE) with the exception of *P. mirabilis* and indole-positive Proteeae, which were categorized

as CRE if doripenem and/or meropenem MIC values were ≥2 mg/L (due to intrinsically elevated imipenem MIC values in these isolates).

Multidrug-resistant *Enterobacteriaceae* was defined as any isolate nonsusceptible when applying the CLSI criteria [7] to ≥1 agent in ≥3 antimicrobial classes, including penicillins combined with a β-lactamase-inhibitor, fluoroquinolones, aminoglycosides, glycylcyclines, and the polymyxins.

Resistance to fluoroquinolones (levofloxacin, ciprofloxacin, and moxifloxacin), aminoglycosides (amikacin, gentamicin, and tobramycin), cephalosporins/monobactams (cefepime, ceftazidime, ceftriaxone, and aztreonam), carbapenems (doripenem, imipenem, and meropenem), and polymyxins (colistin and polymyxin B) was defined as resistance to any agent tested within the class when applying CLSI breakpoint criteria [7] for all agents except for colistin, which used the EUCAST breakpoint [9].

Carbapenemase Screening

Carbapenem-resistant *Enterobacteriaceae* isolates were screened for carbapenemases by polymerase chain reaction (PCR) followed by Sanger sequencing (2007–2015) or whole-genome sequencing and analysis (2016). Multiplex PCR for carbapenemase-encoding genes followed by confirmation by singleplex PCR and sequencing of amplicons was performed as previously described [10]. Whole-genome sequencing was performed using high-quality genomic deoxyribonucleic acid on an MiSeq (Illumina, San Diego, CA) platform targeting a 30× coverage. Sequences were de novo assembled (SPAdes 3.9.0) and queried for the presence of acquired carbapenemases using a curated library and applying criteria of >94% sequencing identity and 40% minimum length coverage [11].

Statistical Analysis

Statistical analysis was performed by the χ^2 test to compare the 1997–2000 period to the 2013–2016 period using SAS 9.4 (SAS Institute Inc., Cary, NC). Prevalence of carbapenemases among CRE isolates from 2007 to 2009 and 2014 to 2016 were compared. These time periods were chosen because the data were more robust and were temporally separated enough to document changes in trends.

RESULTS

Analysis of Extended-Spectrum β-Lactamases, Carbapenem-Resistant *Enterobacteriaceae*, and Multidrug-Resistant Phenotypes

Among 178 825 *Enterobacteriaceae* isolates submitted and tested during the first 20 years of the SENTRY Program, 135 059 were *E. coli*, *K. pneumoniae*, *K. oxytoca*, and *P. mirabilis*, and 24 313 (18.0% of these species) displayed an ESBL phenotype according to the CLSI criteria [7]. The ESBL-phenotype rates varied from 10.3% in the initial survey period (1997–2000) compared with 24.0% in 2013–2016. This increase was statistically significant, as was the increase observed in the ESBL phenotype for all infection sources and geographic regions (Figure 2A and B). Among the infection types, the increase in ESBL phenotype ranged from 10.6% in UTIs to 15.6% among SSSIs (Figure 2A).

The greatest increase in ESBL-phenotype rates was noted for Latin America (22.4% greater in 2013–2016 compared with 1997–2000), followed by Asia-Pacific (17.6% increase), Europe (16.2% increase), and the United States (10.9% increase) (Figure 2B). Isolates exhibiting an ESBL phenotype were mainly *E. coli* (47.5%) and *K. pneumoniae* (43.7%), and the occurrence of these isolates ranged from 3.3% to 15.8% for *E. coli* and 7.1% to 19.4% for *K. pneumoniae* when comparing the initial and later periods of the survey (Figure 2A and B).

Similar to the ESBL-phenotype rates, resistance to a broad-spectrum cephalosporin (ceftazidime, cefepime, or ceftriaxone) or aztreonam among all *Enterobacteriaceae* isolates also increased, and 12.9% of the isolates were resistant to these agents in 1997–2000 compared with 22.4% in 2013–2016 (Figure 3A). Again, these numbers were mainly driven by *E. coli* and *K. pneumoniae* but also *Enterobacter cloacae* species complex (data not shown).

A statistically significant increase in CRE rates was noted over time for the overall isolates and breakdowns by all regions and infection sources (Figure 2A and B). Carbapenem-resistant *Enterobacteriaceae* rates increased from 0.6% in 1997–2000 to 2.9% in 2013–2016 ($P < 0.05$) with gradual increases of 0.8%–0.9% per period since 2005–2008. Carbapenem-resistant *Enterobacteriaceae* rates increased 1.5%, 1.9%, and 2.8% for the United States, Asia-Pacific, and Europe, respectively; however, a more remarkable increase was noted in Latin America, where CRE rates went from 0.8% to 6.4% (5.6% increase). The increase in CRE rates was more pronounced among isolates recovered from patients hospitalized with pneumonia and BSIs (3.3% and 2.5% increases, respectively), but rates also increased 1.8% for SSSIs and 1.2% for UTIs.

Carbapenem-resistant *K. pneumoniae* (CR-KPN) was the main driver of the CRE increase, and this species comprised 71.1% of the CRE isolates. The increase in CR-KPN was statistically significant in all regions and among all infection types. It is interesting to note that the increase in CR-KPN was similar among all infection types (11.4% or 11.5%) except for UTI, which had a slightly lower increase (8.1%) throughout the study. Carbapenem-resistant *K. pneumoniae* increases varied among geographic regions and were higher in Latin America, followed by Europe (Figure 2A and B).

The 4 most common CRE species other than *K. pneumoniae* were *E. cloacae* species complex (9.0% of the CRE), *Serratia marcescens* (5.4%), *E. coli* (4.2%), and *Klebsiella* (formerly *Enterobacter*) *aerogenes* (3.9%). The number of isolates among these species per year was too small for a trend analysis.

Multidrug-resistant rates significantly increased from 7.3% to 15.3%, but variability was observed among different regions

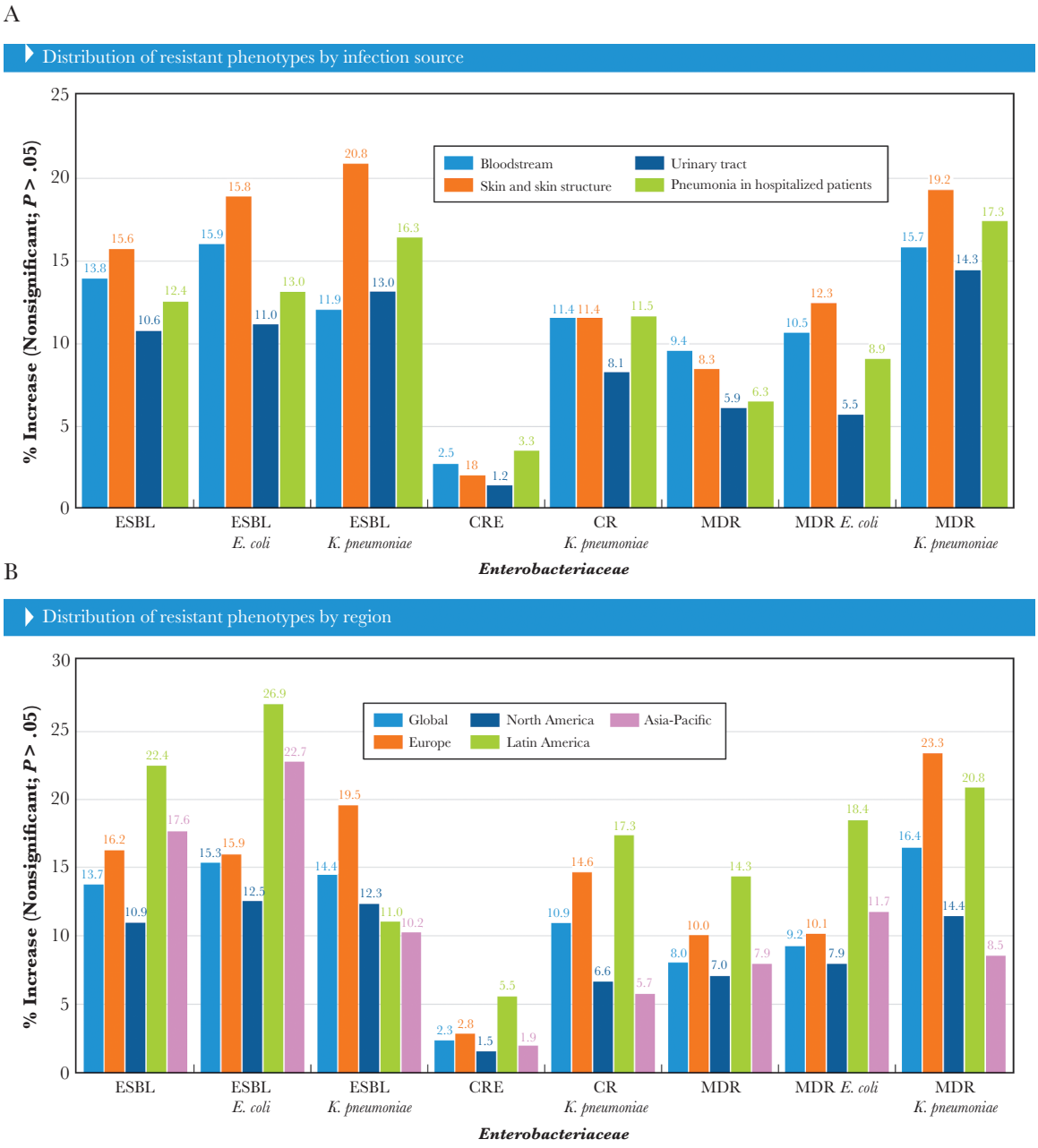


Figure 2. Selected antimicrobial resistance trends for all *Enterobacteriaceae* by (A) infection source and (B) geographic region. Abbreviations: CRE, carbapenem-resistant *Enterobacteriaceae*; ESBL, extended-spectrum β-lactamase; MDR, multidrug-resistant.

and infection sources analyzed. A significant increase in MDR was noted in all regions and all infection sources (Figure 2A and B). The most common MDR species were *K. pneumoniae* (35.2%), *E. coli* (30.2%), *E. cloacae* (9.7%), *P. mirabilis* (6.3%), and *S. marcescens* (5.3%), comprising 86.7% of isolates. A significant increase over time in MDR rates was noted for *K. pneumoniae* (16.4% increase) and *E. coli* (9.2% increase) (Figure 2A and B).

Significant increases in resistance to specific antimicrobial classes were observed among the overall isolate population and main species (Figure 3A and B). Aminoglycoside resistance increased in *E. coli* (7.0% to 18.0%) and *K. pneumoniae* (18.1% to 26.9%) globally and increased overall in North America (4.0% to 11.3%) and Latin America (22.6% to 30.8%) (Figure 3B). Fluoroquinolone resistance increased from 8.8% to 23.3% among the *Enterobacteriaceae* isolates mainly because of *E. coli* (9.5% to 31.4%) and *K. pneumoniae* (7.3% to 27.9%) (Figure 3B). A significant increase ($P < .0001$) in polymyxins (polymyxin B or colistin) resistance rates was noted for *K. pneumoniae* from 2.0% in 2001–2004 when this compound started being tested to 5.5% in 2013–2016 (Figure 3A and B).

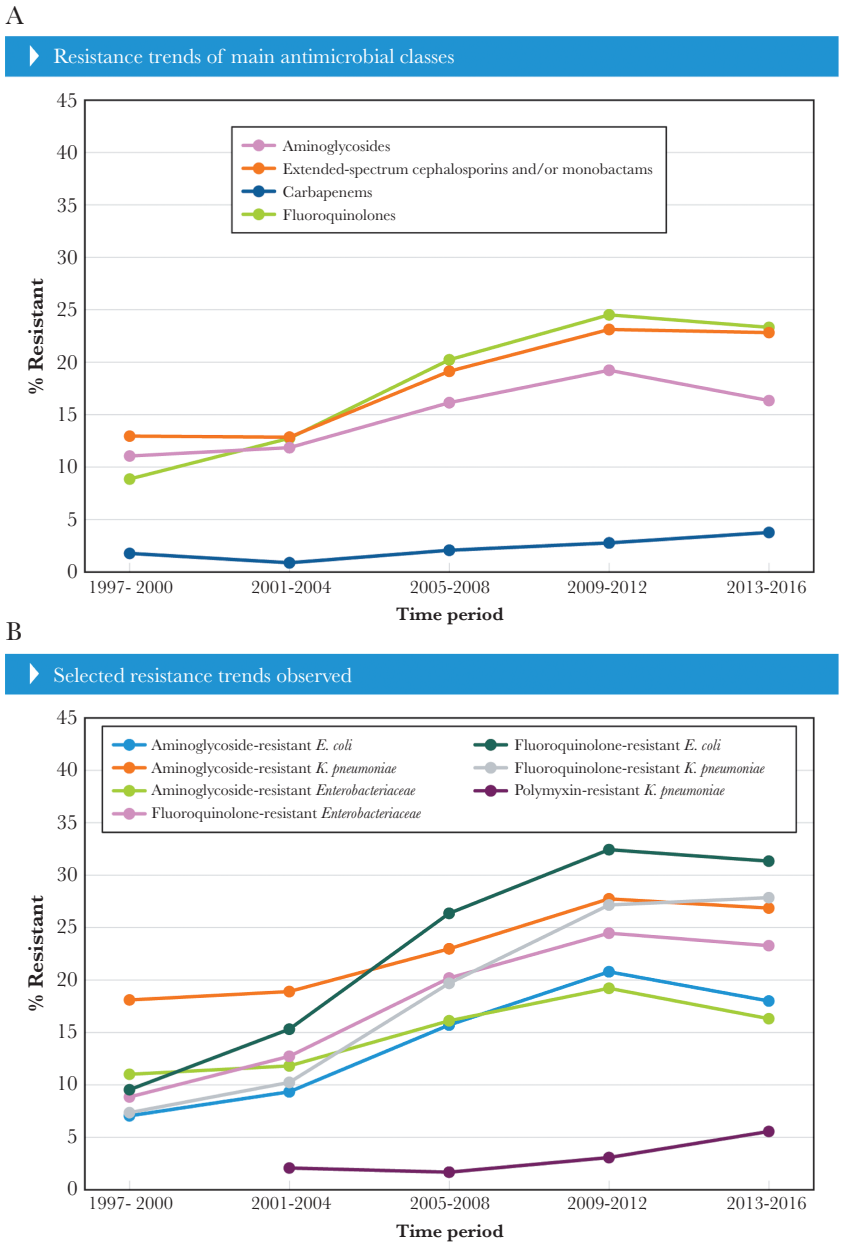


Figure 3. Antimicrobial resistance trends for selected antimicrobial classes of (A) all *Enterobacteriaceae* or (B) bacterial species.

The early analysis of CRE prevalence in the 4 regions demonstrates that an increase from 1.7% to 5.1% occurred in North America from 2004 to 2005, and these rates stayed above 5% until 2016 (Figure 4). Among European countries, an increase in CRE prevalence was noted in 2005 due to the spread of isolates carrying bla_{VIM} in Italy and Greece, but CRE rates were constantly above 5% after 2007 only. For Asia-Pacific and Latin America, CRE rates above 5% were only noted after 2010 and 2008, respectively (Figure 4).

The participation of each country in the survey was not consistent in all cases. Each country's participation and the variations in the ESBL, CRE, and MDR phenotype rates are demonstrated in Figure 5. Among 29 countries that participated in at least 3 defined periods that include 2013–2016, the ESBL rates increased in all countries, except New Zealand, where the ESBL-phenotype rate decreased, and Greece, where the ESBL-phenotype rates were constantly high during the study. Carbapenem-resistant *Enterobacteriaceae* rates increased by less than 2% in 11 of the 29 countries with consistent participation. A moderate CRE increase (5.2% to 11.6%) was observed in 11 other countries, and a remarkable increase in the CRE occurrence was observed in Poland (28.8% difference from the initial and later periods). Carbapenem-resistant *Enterobacteriaceae* rates were unchanged or decreased in 6 countries. Finally, MDR rates increased in all countries, except Greece and Hong Kong.

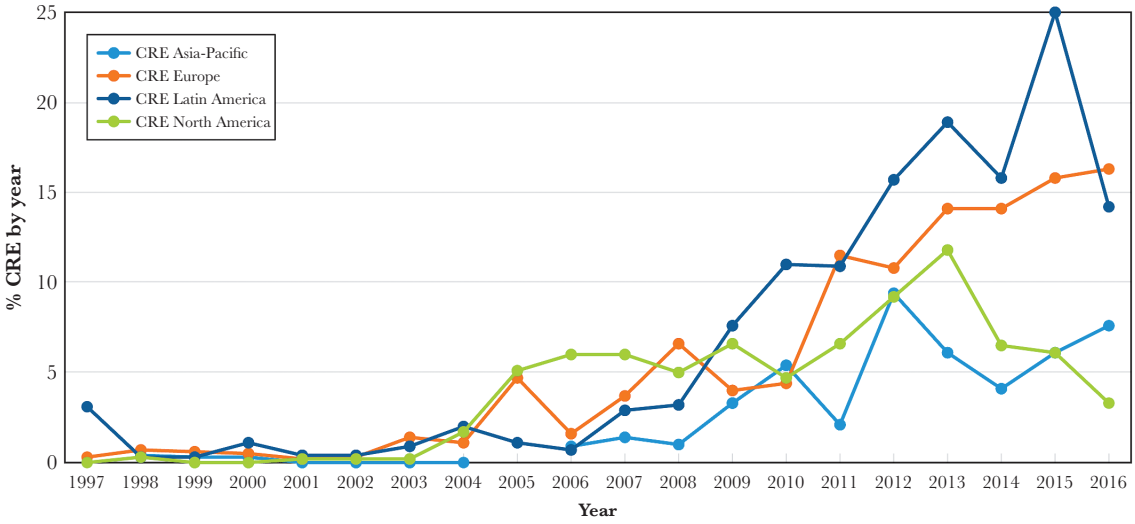


Figure 4. Carbapenem-resistant *Enterobacteriaceae* (CRE) trends over years by region.

Analysis of Carbapenemase Screening Results

Among 2717 CRE isolates, a total of 1788 isolates collected over the years were screened for the presence of carbapenemase genes. Carbapenemase-encoding genes were consistently screened after 2007, and 2007–2009 and 2014–2016 were the periods compared in this analysis. The number of isolates and species observed for each year in these 2 periods are listed in Table 1.

A total of 1298 CRE isolates were observed in these 2 study periods, 374 isolates from 2007 to 2009 and 924 from 2014 to 2016, and 981 isolates were positive for 1 or 2 carbapenemase-encoding genes. Among the carbapenemase-producing *Enterobacteriaceae* (CPE) from 2014 to 2016, *K. pneumoniae* was the most common species (689 of 849), followed by *E. cloacae* (72 of 849). Carbapenemase-producing *Enterobacteriaceae* isolates were detected from 68 sites in both periods, 30 hospitals in 12 countries during 2007–2009 versus 55 hospitals in 25 countries during 2014–2016. It is noteworthy that 58.6% of the CPE isolates were detected in only 10 participating hospitals.

Isolates carrying *K. pneumoniae* carbapenemase (KPC)-encoding genes were the most common in both periods and accounted for 186 (49.7% of the CRE) and 501 (54.2%) isolates in 2007–2009 and 2014–2016, respectively (Table 2). Differently from KPC-harboring isolates that were observed in similar rates in both study periods, a remarkable increase in isolates carrying genes encoding metallo-β-lactamases (MBLs) and oxacillinases (OXAs) was noted. Metallo-β-lactamase-carrying isolates were detected among 4.3% of the isolates from 2007 to 2009 and 12.7% of the isolates from 2014 to 2016 (Table 2). This increase was mainly due to the dissemination of isolates carrying bla_{NDM} that were not observed in 2007–2009 and corresponded to 10.9% of the CRE isolates from 2014 to 2016. Genes encoding IMP and VIM enzymes were observed in 1.9% and 2.4% (7 and 9 isolates) of the isolates from 2007 to 2009 and 0.4% and 1.9% of the isolates from 2014 to 2016 (Table 2). Isolates carrying OXA genes, which were mainly OXA-48 and variants and 1 OXA-23, increased from 4.3% in 2007–2009 to 12.6% in 2014–2016 and were detected mainly in Turkey. The numbers described include only isolates carrying 1 carbapenemase gene; however, 26 isolates harboring 2 carbapenemase genes were noted in 2014–2016. The most common combination (19 of 26 isolates) included genes encoding an OXA-48 plus an MBL, usually bla_{NDM-1}. Other combinations were a KPC plus MBL or OXA-48 (4 and 3 isolates, respectively). In addition, the rates of CRE isolates not carrying carbapenemases decreased remarkably from 41.5% in 2007–2009 to 17.5% in 2014–2016 (Table 2).

DISCUSSION

During the 20 years of the SENTRY Program, we systematically collected and tested >178 000 *Enterobacteriaceae* isolates and demonstrated an increase in cephalosporin-resistance (ESBL phenotype) among *E. coli* and *K. pneumoniae*, CRE, and MDR organisms. Cephalosporin resistance in *E. coli* dramatically increased after the 2005–2008 period (4.6% to 10.4% in 2009–2012). This increase has been noticed by others and has been associated with the worldwide dissemination of the *E. coli* ST131 lineage that carries $bla_{CTX-M-15}$ [12–14]. This observation is corroborated by the increase in fluoroquinolone resistance from 3.6% in 1997–2000 to 11.8% in 2001–2014 and 21.8% in 2005–2008, since it has been established that $bla_{CTX-M-15}$ was acquired by an ST131 *E. coli* isolate that was already resistant to fluoroquinolones [15]. Moreover, some detailed studies were performed using SENTRY Program isolates from US hospitals that confirmed the presence of ST131, $bla_{CTX-M-15}$, and fluoroquinolone resistance in this collection [16–18].

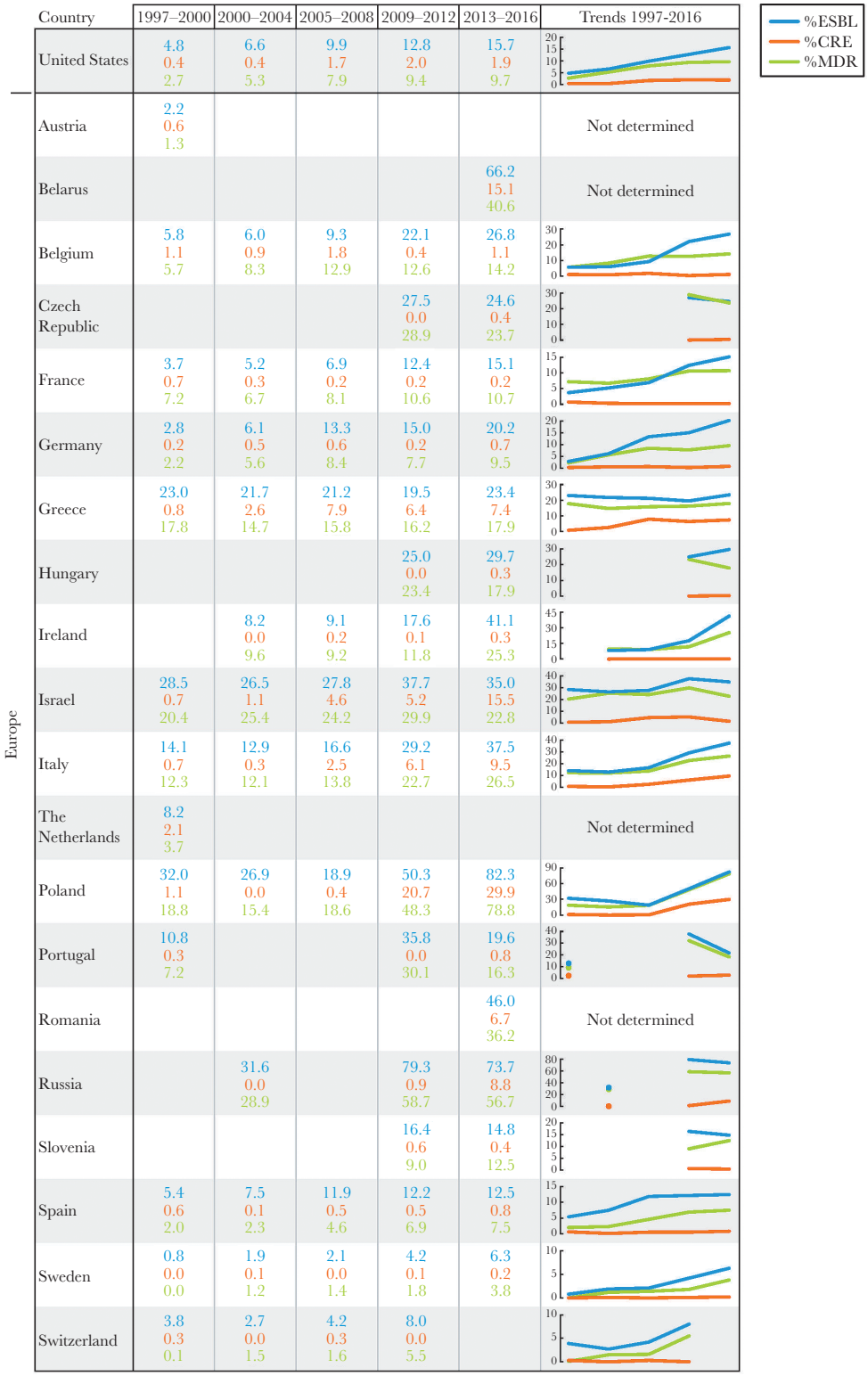
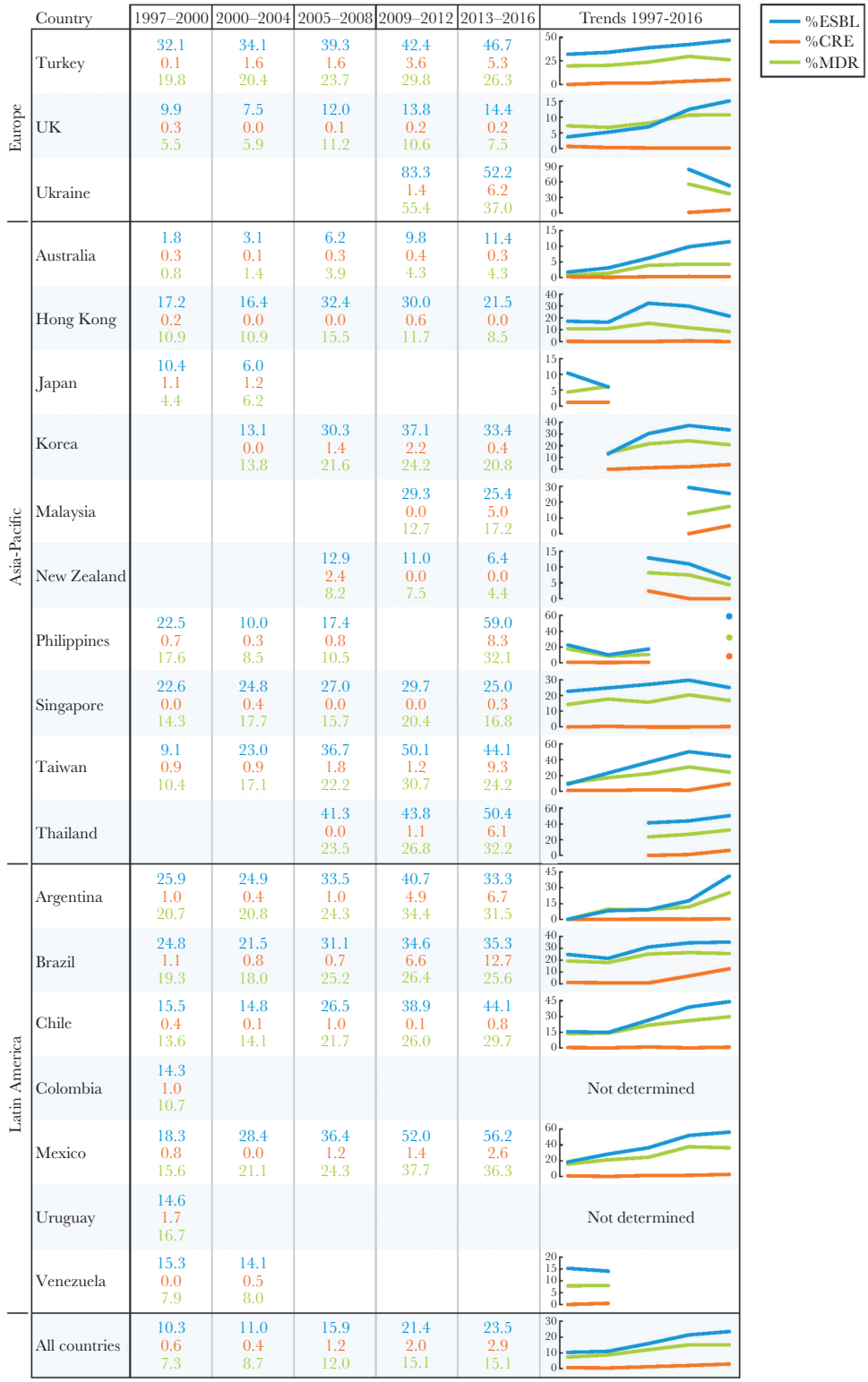


Figure 5. Extended-spectrum β-lactamase (ESBL), carbapenem-resistant *Enterobacteriaceae* (CRE), and multidrug-resistant (MDR) rates among countries participating in the SENTRY Program. (Figure continues on next page)

Among *K. pneumoniae* isolates, the increase in resistance to carbapenems occurred exponentially over the study, and the ESBL-phenotype rates increased almost 15% from 1997–2000 to 2013–2016 (21.7% to 36.1%). We have documented a shift from the predominance of SHV genes encoding ESBLs in *K. pneumoniae* to a majority of isolates carrying $bla_{\text{CTX-M}}$ in US hospitals after



	Country	1997–2000	2000–2004	2005–2008	2009–2012	2013–2016	Trends 1997-2016
Europe	Turkey	32.1 0.1 19.8	34.1 1.6 20.4	39.3 1.6 23.7	42.4 3.6 29.8	46.7 5.3 26.3	
	UK	9.9 0.3 5.5	7.5 0.0 5.9	12.0 0.1 11.2	13.8 0.2 10.6	14.4 0.2 7.5	
	Ukraine				83.3 1.4 55.4	52.2 6.2 37.0	
Asia-Pacific	Australia	1.8 0.3 0.8	3.1 0.1 1.4	6.2 0.3 3.9	9.8 0.4 4.3	11.4 0.3 4.3	
	Hong Kong	17.2 0.2 10.9	16.4 0.0 10.9	32.4 0.0 15.5	30.0 0.6 11.7	21.5 0.0 8.5	
	Japan	10.4 1.1 4.4	6.0 1.2 6.2				
	Korea		13.1 0.0 13.8	30.3 1.4 21.6	37.1 2.2 24.2	33.4 0.4 20.8	
	Malaysia				29.3 0.0 12.7	25.4 5.0 17.2	
	New Zealand			12.9 2.4 8.2	11.0 0.0 7.5	6.4 0.0 4.4	
	Philippines	22.5 0.7 17.6	10.0 0.3 8.5	17.4 0.8 10.5		59.0 8.3 32.1	
	Singapore	22.6 0.0 14.3	24.8 0.4 17.7	27.0 0.0 15.7	29.7 0.0 20.4	25.0 0.3 16.8	
	Taiwan	9.1 0.9 10.4	23.0 0.9 17.1	36.7 1.8 22.2	50.1 1.2 30.7	44.1 9.3 24.2	
	Thailand			41.3 0.0 23.5	43.8 1.1 26.8	50.4 6.1 32.2	
Latin America	Argentina	25.9 1.0 20.7	24.9 0.4 20.8	33.5 1.0 24.3	40.7 4.9 34.4	33.3 6.7 31.5	
	Brazil	24.8 1.1 19.3	21.5 0.8 18.0	31.1 0.7 25.2	34.6 6.6 26.4	35.3 12.7 25.6	
	Chile	15.5 0.4 13.6	14.8 0.1 14.1	26.5 1.0 21.7	38.9 0.1 26.0	44.1 0.8 29.7	
	Colombia	14.3 1.0 10.7					Not determined
	Mexico	18.3 0.8 15.6	28.4 0.0 21.1	36.4 1.2 24.3	52.0 1.4 37.7	56.2 2.6 36.3	
	Uruguay	14.6 1.7 16.7					Not determined
	Venezuela	15.3 0.0 7.9	14.1 0.5 8.0				
	All countries	10.3 0.6 7.3	11.0 0.4 8.7	15.9 1.2 12.0	21.4 2.0 15.1	23.5 2.9 15.1	

Figure 5. Continued

2013 [19], similar to other literature reports [20–22]. The dissemination of carbapenemases that also encode resistance to cephalosporins contributed to the increase in the ESBL-phenotype rates in *K. pneumoniae*.

Another significant finding was the increase in carbapenem resistance overall and dominantly among *K. pneumoniae*. Carbapenem-resistant *Enterobacteriaceae* rates have been observed closely during the past 10 years of the SENTRY Program, and

Table 1. CRE Isolates Recovered in 2 Periods When Isolates Were Screened for Carbapenemase-Encoding Genes

CRE Species	Total Isolates	No. of Isolates by Year					
		2007	2008	2009	2014	2015	2016
Klebsiella pneumoniae	997	75	79	95	224	273	251
Enterobacter cloacae species complex	69	11	19	16	19	24	23
Escherichia coli	52	3	9	7	9	13	11
Serratia marcescens	44	7	5	8	7	12	5
Klebsiella oxytoca	30	6	2	5	4	3	10
Klebsiella aerogenes	27	7	3	4	6	4	3
Citrobacter freundii species complex	9		1		5		5
Proteus mirabilis	6		1		1	2	2
Enterobacter spp.	4	1	2	1			
Raoultella ornithinolytica	3		1			1	1
Pluralibacter gergoviae	2					1	1
Providencia stuartii	2				1		1
Raoultella planticola	2		2				
Serratia spp.	2		2				
Hafnia alvei	1				1		
Morganella morganii	1			1			
Pantoea agglomerans	1	1					
Raoultella spp.	1						1
Total	1298	111	126	137	277	333	314

Abbreviation: CRE, carbapenem-resistant *Enterobacteriaceae*.

genetic analysis of these isolates has been performed to identify the occurrence of carbapenemases [10, 23]. Not only was a significant increase in the CRE rates (0.6% to 2.9%) detected worldwide and in all infection sources monitored, but a dramatic change in the epidemiology of the carbapenemase genes was observed. These changes have also been reported in the literature, and our data support the observations that KPC-encoding genes continue to prevail in several geographic areas and that the dissemination of isolates carrying genes encoding NDM and OXA-48 variants have contributed to the increasing CRE rates in the period after 2012 [24]. It is noteworthy that the CRE isolates not carrying carbapenemases decreased over time, and this trend should be monitored closely after introducing the new β-lactam/β-lactamase inhibitor combinations that display activity against serine-carbapenemase-producing isolates.

Resistance to other antimicrobial classes important for treating infections caused by *Enterobacteriaceae* also increased, and a remarkable increase of MDR isolates was also documented. Multidrug-resistant *Enterobacteriaceae* often are secondarily important because MDR nonfermentative organisms are more common and challenging for patient management [3]; however, *Enterobacteriaceae* isolates resistant to >3 antimicrobial classes greatly limit therapeutic options and have become more common regardless of the infection source or geographic region and are observed among the most common *Enterobacteriaceae* species.

Our data demonstrated increasing resistance trends that the medical and scientific communities have been monitoring over the years. Since 1995, when the American Society for Microbiology assembled a task force to address the issue of the increase in antimicrobial resistance, surveillance initiatives have been highlighted as an important tool for assisting in this endeavor [25]. Different surveillance programs fulfill the interests of prescribing physicians, microbiologists, infection control specialists, public health and regulatory authorities, and the pharmaceutical industry, and all have well recognized biases or inaccuracies [26, 27]. Nevertheless, these surveys provide important information that allows the identification of trends in pathogen incidence and antimicrobial resistance. The importance of surveillance has been continuously reinforced by several organizations and expert panels recognize that surveillance in local, regional, and global levels is essential to address antimicrobial resistance issues. Furthermore, global surveillance is recognized as providing (1) early warnings of emerging threats and (2) data to identify and act on long-term trends. Knowing local and regional resistance levels provides important information for empiric antimicrobial therapy decision options (https://amr-review.org).

CONCLUSIONS

The SENTRY Program is the largest and longest-operating worldwide surveillance initiative that comprises the most important human infections, provides consistently high-quality data, and evaluates timely trends in antimicrobial resistance. This program does not address important issues such as antibiotic use, how antibiotic therapy benefits patients directly and indirectly, or clinical outcomes; however, it addresses issues that

Table 2. Carbapenemase Genes Detected Among CRE Isolates From 2007 to 2009 and 2014 to 2016

		2007–2009		2014–2016	
Carbapenemase Group/Carbapenemase	No. of Isolates From Both Periods	n	%	n	%
MBL	133	16	4.3	117	12.7
IMP	11	7	1.9	4	0.4
IMP-1	3	3	0.8		
IMP-4	3	1	0.3	2	0.2
IMP-8	1			1	0.1
IMP-18	1	1	0.3		
IMP-26	2	2	0.5		
IMP-64	1			1	0.1
NDM	95			95	10.3
NDM-1	88			88	9.5
NDM-7	5			5	0.5
NDM-9	2			2	0.2
VIM	27	9	2.4	18	1.9
VIM-1	21	7	1.9	14	1.5
VIM-2	1	1	0.3		
VIM-4	3			3	0.3
VIM-5	1			1	0.1
VIM-23	1	1	0.3		
Serine carbapenemases	690	187	50.0	503	54.4
KPC	687	186	49.7	501	54.2
KPC-2	249	52	13.9	197	21.3
KPC-3	336	42	11.2	294	31.8
KPC-4	2			2	0.2
KPC-6	1			1	0.1
KPC-12	1			1	0.1
KPC-17	1			1	0.1
KPC-20	1			1	0.1
KPC-like[a]	96	92	24.6	4	0.4
SME	3	1	0.3	2	0.2
SME-2	1	1	0.3		
SME-4	2			2	0.2
OXA	132	16	4.3	116	12.6
OXA-23	1			1	0.1
OXA-48	128	16	4.3	112	12.1
OXA-163	1			1	0.1
OXA-232	1			1	0.1
OXA-244	1			1	0.1
Double carbapenemases	26			26	2.8
KPC+MBL	4			4	0.4
KPC-2, NDM-1	1			1	0.1
KPC-3, NDM-1	1			1	0.1
KPC-3, VIM-1	1			1	0.1
KPC-17, NDM-1	1			1	0.1
MBL+OXA-48	19			19	2.1
NDM-1, OXA-232	10			10	1.1
NDM-1, OXA-48	5			5	0.5
NDM-5, OXA-232	2			2	0.2
VIM-1, OXA-48	2			2	0.2
KPC+OXA-48	3			3	0.3
KPC-3, OXA-48	3			3	0.3
Negative	281	124	33.2	157	17.0
Not tested	36	31	8.3	5	0.5

Abbreviations: CRE, carbapenemase-resistant *Enterobacteriaceae*; IMP, imipenemase metallo-β-lactamase; KPC, *K. pneumoniae* carbapenemase; MBL, metallo-β-lactamase; NDM, New Delhi metallo-β-lactamase; OXA, oxacillinase.

[a]Amplicon sequencing was not performed. Isolates were tested using a multiplex reaction and reamplified using singleplex.

have been a great concern to the infectious diseases and clinical microbiology communities for many years.

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