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Changes in the Frequencies of β-Lactamase Genes among *Enterobacteriaceae* Isolates in U.S. Hospitals, 2012 to 2014: Activity of Ceftazidime-Avibactam Tested against β-Lactamase-Producing Isolates

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Among 15,588 *Enterobacteriaceae* isolates collected in 63 U.S. hospitals from 2012 to 2014, 2,129 (13.7%) displayed an extended-spectrum β-lactamase (ESBL) phenotype. These rates were similar over time (13.2 to 13.9%); however, differences among *Escherichia coli* (12.7 and 15.1% in 2012 and 2014; $P = 0.007$) and *Klebsiella pneumoniae* (18.9 and 15.5% in 2012 and 2014; $P = 0.006$) were noted when comparing 2014 and 2012. Carbapenem-resistant *Enterobacteriaceae* (CRE) (2.3 and 1.8%) and carbapenem-resistant *K. pneumoniae* (6.8 and 5.1%; $P = 0.003$) rates were lower in 2014 than in 2012. Isolates carrying $bla_{\text{CTX-M-15}}$-like genes were stable (42.1 to 42.4%), but a decrease among *E. coli* isolates (59.1 and 49.7%; $P = 0.008$) and an increase among *K. pneumoniae* isolates (32.7 and 41.2%; $P = 0.022$) in 2014 were observed. Isolates carrying bla_{KPC} (304) decreased over the years (16.5 and 10.9%; $P = 0.008$), mainly due to the decrease in *K. pneumoniae* isolates harboring bla_{KPC} ($n = 285$; 35.6 and 28.4%; $P = 0.041$) in hospitals in the Mid-Atlantic and South Atlantic regions, where these isolates were highly prevalent during 2012 and 2013. Isolates carrying $bla_{\text{CMY-2}}$-like and $bla_{\text{CTX-M-14}}$-like genes increased (8.2 and 11.9% and 9.1 and 12.9%, respectively; $P = 0.04$ for both), and those producing bla_{SHV} ESBL decreased (24.9 and 12.7%; $P < 0.001$) over the studied years, due to a decreased occurrence of the enzymes among *K. pneumoniae* isolates. Other enzymes were detected in smaller numbers of isolates, including four *K. pneumoniae* isolates carrying $bla_{\text{NDM-1}}$ metallo-β-lactamase (two in 2012 and two in 2014). Ceftazidime-avibactam, a recently approved β-lactamase inhibitor combination, was very active against the ESBL phenotype isolates ($MIC_{50/90}$, 0.12 and 1 μg/ml; 99.7% susceptible) and CRE strains ($MIC_{50/90}$, 0.5 and 2 μg/ml; 98.5% susceptible) that displayed elevated MIC values for many comparator agents. In conclusion, significant changes were noted in the frequencies of isolates harboring various β-lactamases among U.S. hospitals between 2012 and 2014 that will require continued monitoring.

Enterobacteriaceae isolates producing extended-spectrum β-lactamases (ESBLs) or carbapenemases (carbapenem-resistant *Enterobacteriaceae* [CRE]) are usually resistant to most or all β-lactam agents. These isolates often coharbor resistance mechanisms to other antimicrobial classes that are carried on mobile genetic elements, which promote the dissemination of these resistance genes (1, 2). These isolates pose a challenge for clinical microbiology laboratories, infection control practitioners, and clinicians due to the difficulties in detecting, containing, and treating infections caused by these emerging organisms. Monitoring the occurrence and prevalence of the isolates is prudent; however, very few studies in U.S. hospitals report on the prevalence and characteristics of broad collections of *Enterobacteriaceae* isolates producing the enzymes.

We recently reported on the prevalence of common β-lactamase genes among isolates collected in over 70 U.S. hospitals during 2012 (3) and 2013 (4) that displayed a positive ESBL phenotype according to the Clinical and Laboratory Standards Institute (CLSI) MIC-based epidemiological criteria. During these and other published studies, it has been demonstrated that the β-lactamase prevalence scenario in the United States differs from that in other countries regarding the occurrence and distribution of β-lactamase-producing isolates and enzyme types (3). A decade ago, isolates carrying $bla_{\text{CTX-M}}$ were considered endemic in nosocomial and community settings in countries in Europe and Asia (5), but the first studies showing the dissemination of isolates harboring $bla_{\text{CTX-M}}$ in the United States date from 2007 (6, 7). After the initial reports, a rapid spread of isolates harboring $bla_{\text{CTX-M-15}}$-like and $bla_{\text{CTX-M-14}}$-like genes was observed in U.S. hospitals, and currently, the rates are becoming more similar to those observed in other nations (8).

The emerging scenario in U.S. hospitals is also different regarding the presence of carbapenemases. Isolates carrying bla_{KPC} that were first described in North Carolina and later in New York City (9), became endemic in the latter geographic region and surrounding areas. More recently, isolates producing these serine-carbapenemases have been detected throughout the country (3, 4). Furthermore, metallo-β-lactamase (MBL)-producing isolates have been uncommon in U.S. hospitals; however, this could rapidly change with the dissemination of *Enterobacteriaceae* isolates harboring $bla_{\text{NDM-1}}$, which have been reported in 22 states (10).

In this study, we assess the occurrence of β-lactamase-producing isolates collected in 69 U.S. hospitals during 2014 and evaluate trends of these enzymes in 63 institutions participating in all three consecutive years of surveillance, including 2012 and 2013, which

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TABLE 1 Results of screening for β-lactamase genes/families among 782 ESBL phenotype isolates collected during 2014 in 69 U.S. hospitals

β-Lactamase gene/family	No. of positive results[a]				
	Overall (782)[b]	*E. coli* (409)	*K. oxytoca* (58)	*K. pneumoniae* (284)	*P. mirabilis* (31)
CRE (including MBL)					
bla_{KPC}	90	4	3	83	
bla_{NDM-1}	2	1		1	
ESBL					
$bla_{CTX-M-15}$-like	326	204	1	113	8
bla_{SHV} ESBL	106	9	10	87	
$bla_{CTX-M-14}$-like	100	84		13	3
bla_{TEM} ESBL	15	11		1	3
$bla_{CTX-M-8}$-like	2			1	1
$bla_{CTX-M-2}$-like	1				1
Transferrable AmpC					
bla_{CMY-2}-like	92	74		7	11
bla_{FOX}-like	7	2	2	3	
bla_{DHA}-like	3	2		1	
$bla_{ACT/MIR}$	1	1			
Non-ESBL					
bla_{TEM} WT	360	171	8	166	15
bla_{SHV} WT	271	8		263	
Negative result	65	17	43	2	3

[a] Isolates can be positive for more than one test/gene.
[b] Number tested.

have been previously reported as parts of larger collections (3, 4). Additionally, we evaluated the activities of ceftazidime-avibactam and comparator antimicrobial agents against the isolates producing β-lactamases.

MATERIALS AND METHODS

Bacterial isolates. A total of 5,771 clinical isolates, including *Escherichia coli* (n = 2,813), *Klebsiella pneumoniae* (n = 1,832), *Klebsiella oxytoca* (n = 502), and *Proteus mirabilis* (n = 624) clinical isolates, were collected as part of the International Network for Optimal Resistance Monitoring (INFORM) program in 69 U.S. medical centers during 2014. Only one isolate per patient infection episode was included in the study. Species identification was confirmed when necessary by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) using the Bruker Daltonics MALDI Biotyper (Billerica, MA) following the manufacturer's instructions.

For the multiple-year comparison, 15,588 clinical isolates of *E. coli* (n = 7,688), *K. pneumoniae* (n = 4,845), *K. oxytoca* (n = 1,250), and *P. mirabilis* (n = 1,805) from 63 U.S. hospitals participating in a surveillance study from 2012 to 2014 were analyzed. ESBL phenotype isolates collected from 2012 to 2014 were recovered from bloodstream infections (n = 340), intra-abdominal infections (n = 154), pneumonia in hospitalized patients (n = 556), skin/soft tissue infections (n = 468), urinary tract infections (n = 504), and other or unknown sites (n = 107).

Antimicrobial susceptibility testing. Isolates were susceptibility tested using the reference broth microdilution method as described by the CLSI (11). The categorical interpretations for all antimicrobials were those found in CLSI M100-S25 (2015), and quality control (QC) was performed using *E. coli* ATCC 25922 and 35218, *K. pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853 (12). All QC results were within the ranges published in the CLSI document (12).

Screening for β-lactamases. Isolates displaying an ESBL phenotype (MIC, >1 μg/ml for aztreonam and/or ceftazidime and/or ceftriaxone [12]) were tested for β-lactamase-encoding genes using the microarray-based Check-MDR CT101 assay kit (Check-Points, Wageningen, Netherlands). The assay was performed according to the manufacturer's instructions. The kit has the capability to detect bla_{CTX-M} group 1, 2, 8 plus 25, and 9; bla_{TEM} wild type (WT) and ESBL; bla_{SHV} WT and ESBL; bla_{ACC}; $bla_{ACT/MIR}$; bla_{CMY-2}-like; bla_{DHA}; bla_{FOX}; bla_{KPC}; and bla_{NDM-1}-like genes The most common amino acid alterations that expand the spectrum of TEM and SHV enzymes are detected by the assay and include E104K, R164S/H, and G238S for TEM and G238A/S and E240K for SHV. Validation of the assay against U.S. isolates was performed previously (8).

All isolates displaying a ceftazidime-avibactam MIC value of >4 μg/ml were screened for the presence of metallo-β-lactamase- and serine-carbapenemase-encoding gene families (bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{KPC}, bla_{OXA-48}, bla_{GES}, bla_{IMI}, bla_{NMC-A}, and bla_{SME}) by PCR as previously described (13). Amplicons were sequenced on both strands, and the results were analyzed using the Lasergene software package (DNASTAR, Madison, WI). The amino acid sequences were compared with those available in the NCBI database using BLAST.

Statistical analysis and definitions. Statistical analysis was performed by Fisher's exact test comparing 2012 and 2014 rates using EpiInfo 7 (Centers for Disease Control and Prevention, Atlanta, GA). CRE were those isolates displaying imipenem and/or meropenem MIC values of >2 μg/ml (14).

RESULTS

Occurrence of β-lactamases in U.S. hospitals during 2014. A total of 782 *Enterobacteriaceae* isolates collected during 2014 displayed an ESBL phenotype: 409 *E. coli* (14.5% of the overall samples for the species), 284 *K. pneumoniae* (15.6%), 58 *K. oxytoca* (11.5%), and 31 *P. mirabilis* (5.0%) isolates (Table 1).

Isolates carrying $bla_{CTX-M-15}$-like genes (including $bla_{CTX-M-1}$, $bla_{CTX-M-15}$, and $bla_{CTX-M-3}$) were the most prevalent. A total of 326 isolates yielded positive results for $bla_{CTX-M-15}$-like genes among all four species tested, often in combination with other

β-lactamase-encoding genes/families. Additionally, 100 isolates carried $bla_{CTX\text{-}M\text{-}14}$-like family genes—$bla_{CTX\text{-}M\text{-}9}$ and $bla_{CTX\text{-}M\text{-}27}$, among others. The majority of these isolates were *E. coli* ($n = 84$), but *K. pneumoniae* ($n = 13$) and *P. mirabilis* ($n = 3$) were also detected.

Carbapenemase-encoding genes were observed among 92 isolates. bla_{KPC} was detected among 90 isolates, the majority being *K. pneumoniae* (83 isolates), but also in *E. coli* ($n = 4$) and *K. oxytoca* ($n = 3$) isolates. $bla_{NDM\text{-}1}$ was detected in two isolates, a *K. pneumoniae* isolate from New York and an *E. coli* isolate from California, with results confirmed by DNA sequencing.

bla_{SHV} genes encoding enzymes with an extended spectrum of activity (bla_{SHV} ESBL) were detected among 106 (13.6%) isolates, and the vast majority of the isolates were *K. pneumoniae* (87/106; 82%). bla_{TEM} genes encoding an ESBL variant were observed in 15 isolates, including three bacterial genera/species (Table 1). Other ESBL genes detected were $bla_{CTX\text{-}M\text{-}8}$-like (two isolates and two genera/species) and $bla_{CTX\text{-}M\text{-}2}$-like (one isolate) genes.

Additionally, transferable cephalosporinases (plasmidic AmpCs) were detected among 103 (13.2%) isolates, and 92 (89.3%) strains displayed a positive result for $bla_{CMY\text{-}2}$-like genes (74 *E. coli*, 7 *K. pneumoniae*, and 11 *P. mirabilis*) (Table 1). Other AmpC genes detected were bla_{FOX} (seven isolates and three bacterial genera/species), bla_{DHA} (three isolates and two bacterial species), and $bla_{ACT/MIR}$ (one isolate each). A total of 54 isolates did not carry the screened genes encoding ESBL ($bla_{CTX\text{-}M}$, bla_{SHV} ESBL, or bla_{TEM} ESBL), transferable AmpC, or carbapenemases, and these isolates yielded only positive results for narrow-spectrum bla_{SHV} and/or bla_{TEM}. Additionally, 65 isolates displayed negative results for all β-lactamase genes/families screened, which were mostly *K. oxytoca* (43 isolates) (data not shown).

Comparison of β-lactamase occurrences from 2012 to 2014. A total of 2,129 (13.7% of targeted species) isolates met the MIC-based ESBL criteria during the 2012-2014 period in hospitals participating in all years of surveillance. This total included 1,048 *E. coli* (13.6% for this species), 843 *K. pneumoniae* (17.4%), 135 *K. oxytoca* (10.8%), and 103 *P. mirabilis* (5.7%) isolates. The overall ESBL rates were similar over time and were 13.8, 13.2, and 13.9% in 2012, 2013, and 2014, respectively (comparing 2012 to 2014: $P = 0.841$; odds ratio [OR], 0.988; 95% confidence interval [CI], 0.883 to 1.105) (Fig. 1); however, an increase in the ESBL phenotype among *E. coli* isolates from 12.7 to 15.1% ($P = 0.007$; OR, 0.817; 95% CI, 0.695 to 0.959) and a decrease in the ESBL phenotype among *K. pneumoniae* isolates from 18.9 to 15.5% ($P = 0.006$; OR, 1.264; 95% CI, 1.054 to 1.517) was documented (Fig. 1).

Differences in the ESBL phenotype rates among census regions were noted, and a significant decrease from 20.8 to 9.2% ($P < 0.001$; OR, 2.582; 95% CI, 1.792 to 3.722) in the ESBL phenotype rates between 2012 and 2014 was documented in the South Atlantic region (Fig. 2). Additionally, a minor decrease in ESBL phenotype isolates was noted in the New England region ($P = 0.086$; OR, 1.484; 95% CI, 0.881 to 2.502) (Fig. 2). In both census regions, the overall decrease was caused by the reduced occurrence of *E. coli* isolates displaying an ESBL phenotype in the last year of the study (16.7 to 10.3% in the South Atlantic and 11.5 to 7.5% in the New England regions). Conversely, a significant increase in ESBL phenotype rates from 2012 to 2014 was observed in the West North Central (5.3 to 9.1%) ($P = 0.016$; OR, 0.563; 95% CI, 0.338 to 0.936), East South Central (9.4 to 14.1%) ($P = 0.014$; OR, 0.632; 95% CI, 0.425 to 0.940), and Pacific (11.4 to 16.9%) ($P = 0.003$; OR, 0.628; 95% CI, 0.452 to 0.869) regions (Fig. 2). An increased frequency during 2014 was noted for ESBL phenotype *E. coli* in the same three regions (5.8 to 14.2%, 8.9 to 16.7%, and 12.9 to 19.1% for the West North Central, East South Central, and Pacific regions, respectively), in *K. pneumoniae* for the East South Central (11.0 to 14.4%) and Pacific (11.6 to 14.0%) regions, and in *K. oxytoca* (9.0 to 17.9%) and *P. mirabilis* (5.4 to 14.8%) in the Pacific region (data not shown).

CRE represented 2.1% of the total isolates during the study period, and a slight but not statistically significant decline was observed from 2012 (2.3%) to 2014 (1.8%) ($P = 0.110$; OR, 1.253; 95% CI, 0.953 to 1.647) (Fig. 1). Among the 326 CRE organisms, 304 (93.3%) were *K. pneumoniae*, and the remaining isolates were *E. coli* ($n = 15$) and *K. oxytoca* ($n = 7$). The frequency of the carbapenem resistance phenotype within *K. pneumoniae* was significantly higher in 2012 (6.8%) than in 2014 (5.1%). Other carbapenem-resistant (CR) organisms occurred in limited numbers each year; however, carbapenem-resistant *E. coli* isolates displayed a steady increase over the study interval (two, five, and eight isolates in 2012, 2013, and 2014, respectively).

Among U.S. census regions, a statistically significant decline in CRE rates was noted in the Mid-Atlantic (9.2 to 6.0%) ($P = 0.018$; OR, 1.593; 95% CI, 1.050 to 2.419) and South Atlantic (3.1 to 0.9%) ($P = 0.009$; OR, 3.494; 95% CI, 1.260 to 9.685) (Fig. 2) regions when comparing 2012 to 2014 rates, although a small number of isolates were present in the South Atlantic region in 2012, 2013, and 2014. In both census regions, a decrease among carbapenem-resistant *K. pneumoniae* isolates was observed; however, in the Mid-Atlantic region (24.7 to 19.9%; not statistically significant), a reduction in carbapenem-resistant *K. oxytoca* (5.7 to 2.9%) also contributed to the regional decline in CRE rates.

Among the most common β-lactamase-encoding genes, $bla_{CTX\text{-}M\text{-}15}$-like genes occurred among 295, 297, and 326 isolates in 2012, 2013, and 2014, respectively. Although the overall prevalence of these isolates was stable (42.4, 42.2, and 42.1% in 2012, 2013, and 2014, respectively) ($P = 0.474$; OR, 1.013; 95% CI, 0.821 to 1.250), a decrease in the frequency of *E. coli* isolates carrying $bla_{CTX\text{-}M\text{-}15}$-like genes (59.1 to 49.7%) ($P = 0.008$; OR, 1.460; 95% CI, 1.081 to 1.972) and an increase in the frequency of the enzyme family among *K. pneumoniae* isolates (32.7 to 41.2%) ($P = 0.022$; OR, 0.692; 95% CI, 0.490 to 0.976) were documented in 2014. The 2014 decrease in the occurrence of *E. coli* isolates carrying $bla_{CTX\text{-}M\text{-}15}$-like genes was noted in different U.S. census regions, including the New England (56.5 and 38.5% in 2012 and 2014, respectively), Mid-Atlantic (62.3 and 49.4%), East North Central (66.0 and 46.8%), South Atlantic (70.3 and 37.9%), and Mountain (55.6 and 42.9%) regions (data not shown); however, these differences were either not statistically significant or the number of isolates was not large enough for meaningful interpretation.

A decrease in isolates harboring bla_{KPC} was observed in the study years: 16.5% of ESBL phenotype isolates carried bla_{KPC} in 2012, 15.5% in 2013, and only 10.9% in 2014 ($P = 0.008$; OR, 3.868; 95% CI, 1.188 to 2.192). This trend was mainly due to the decrease of *K. pneumoniae* isolates carrying bla_{KPC} from 35.6% in 2012 and 36.8% in 2013 to 28.4% in 2014 ($P = 0.041$; OR, 1.3; 95% CI, 0.975 to 1.995). The largest decline in bla_{KPC}-carrying isolates was in the Mid-Atlantic region (68.4 to 52.5%) ($P = 0.043$; OR, 1.1; 95% CI, 0.972 to 3.917) (data not shown). *E. coli* and *K. oxytoca* isolates harboring bla_{KPC} were detected in small numbers in

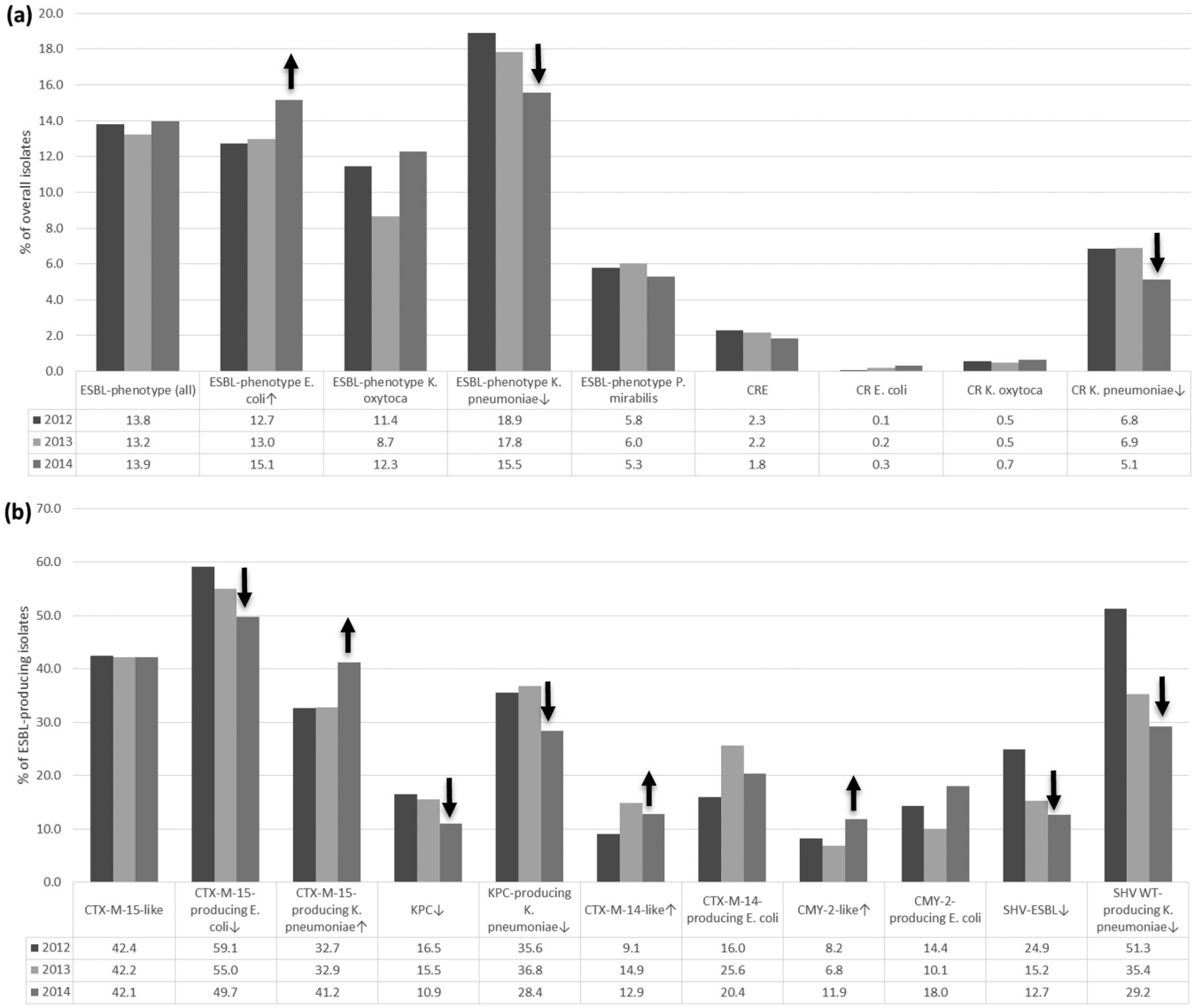

FIG 1 (a) Percent occurrences of ESBL phenotype and CRE isolates collected in 63 U.S. hospitals from 2012 to 2014 among all isolates collected during the period. (b) Percent occurrences of the most common β-lactamase genes among ESBL phenotype isolates. Statistically significant shifts upward (↑) or downward (↓) are indicated.

all 3 years (two to four isolates each per year) and did not have a significant impact on the differences observed.

A significant increase in the percentage of isolates carrying $bla_{\text{CTX-M-14}}$-like genes did occur from 2012 (9.1%) to 2014 (12.9%) ($P = 0.042$; OR, 0.700; 95% CI, 0.499 to 0.982) (Fig. 1), although higher rates of this family of genes was noted in 2013 (14.9%). $bla_{\text{CTX-M-14}}$-like genes were most prevalent among *E. coli* isolates, and an increase in more recent years (25.6 and 20.4% in 2013 and 2014) was noted compared to 2012 (16.0%); although the difference between 2012 and 2014 was not statistically significant ($P = 0.081$; OR, 0.744; 95% CI, 0.503 to 1.099), higher rates were observed in 2013 ($P = 0.001$; OR, 0.551; 95% CI, 0.374 to 0.811) than in 2012. *E. coli* isolates carrying $bla_{\text{CTX-M-14}}$-like genes were also increasingly observed in four of the nine census regions—New England (13.0 to 23.1%), Mid-Atlantic (16.4 to 31.0%), East North Central (10.0 to 14.9%), and East South Central (15.0 to 27.0%)—but lower rates were noted in the West North Central region (26.7 to 13.5% in 2012 and 2014, respectively).

Isolates harboring $bla_{\text{CMY-2}}$-like genes increased from 2012 (8.2%) to 2014 (11.9%) ($P = 0.042$; OR, 0.685; 95% CI, 0.482 to 0.974), but only 6.8% of all 2013 ESBL phenotype isolates carried the gene encoding the enzyme. Among the organisms carrying $bla_{\text{CMY-2}}$-like genes, *E. coli* isolates displayed a modestly increased rate compared to the overall ESBL phenotype population: 14.4, 10.1, and 18.0% in 2012, 2013, and 2014, respectively ($P = 0.115$; OR, 0.763; 95% CI, 0.507 to 1.147) (Fig. 1), and these increased rates were observed in the New England (13.0 to 30.8%), East North Central (6.0 to 12.8%), West North Central (26.7 to 35.1%), South Atlantic (8.1 to 31.0%), and East South Central (5.0 to 10.8%) regions.

Interestingly, an important decrease in the percentage of iso-

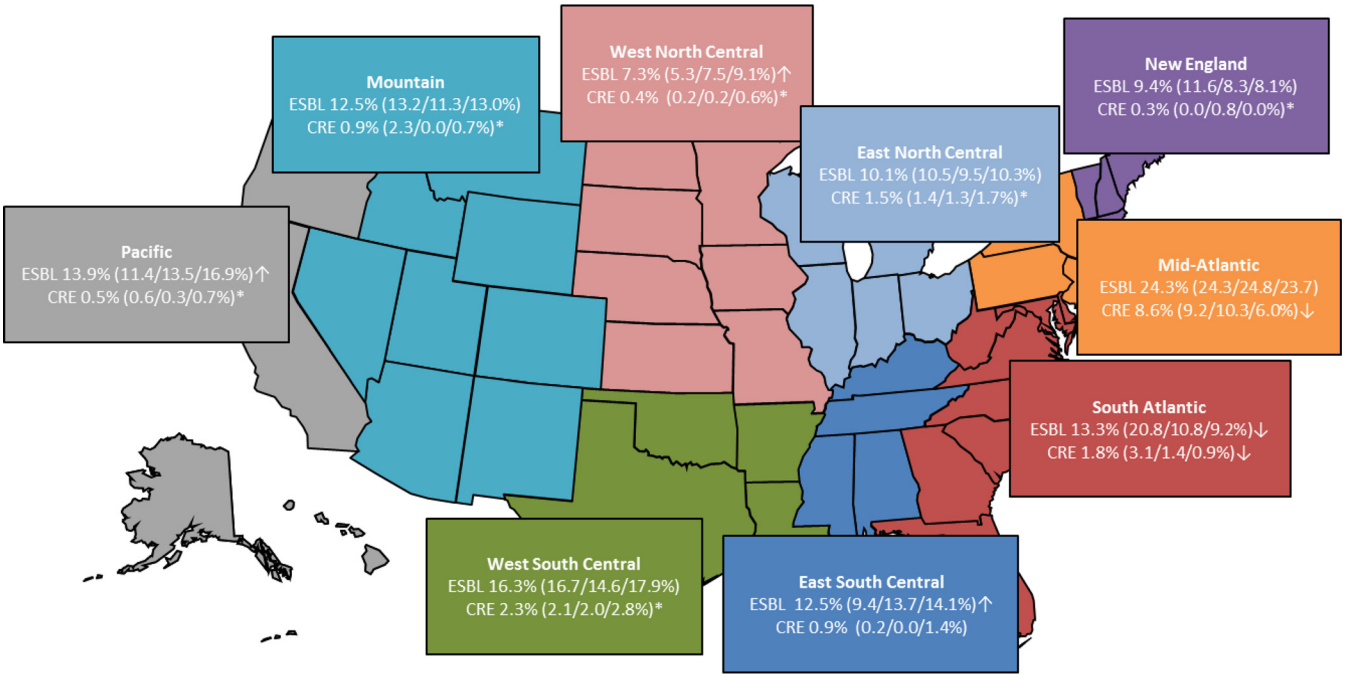

FIG 2 Occurrence and changes in ESBL phenotype and CRE isolates (overall percentages) among 15,588 isolates collected from 63 U.S. hospitals during 2012 to 2014. ↑, statistically significant increase; ↓, statistically significant decrease; *, number too small to calculate.

lates carrying bla_{SHV} ESBL-producing organisms from 2012 (24.9%) to 2014 (12.7%) ($P < 0.001$; OR, 2.274; 95% CI, 1.723 to 3.000) was discovered, and it was caused by a decline in bla_{SHV} ESBL occurrence among *K. pneumoniae* isolates from 51.3% in 2012 to 29.2% in 2014 ($P < 0.001$; OR, 2.557; 95% CI, 1.801 to 3.629). It is noteworthy that a decrease in the percentage of bla_{SHV} ESBL enzymes was noted in seven of the census regions—New England (53.8 and 20.0% in 2012 and 2014, respectively), Mid-Atlantic (50.6 and 33.9%), East North Central (46.7 and 22.5%), East South Central (50.0 and 23.1%), West South Central (42.4 and 37.5%), Mountain (55.6 and 11.1%), and Pacific (69.6 and 25.9%)—while rates in the remaining regions were stable.

Differences in the relative frequencies of other β-lactamase-encoding genes were also observed in the study period. However, the number of positive isolates was small, and statistical analysis could not be performed.

Antimicrobial activities of ceftazidime-avibactam and comparator agents. Ceftazidime-avibactam was very active when tested against the 2,129 ESBL phenotype isolates ($MIC_{50/90}$, 0.12/1 μg/ml) (Table 2) collected in U.S. hospitals during 2012 to 2014, and the compound inhibited nearly all (99.7%) of the isolates, applying the U.S. FDA breakpoints (15). Ceftazidime alone, ceftriaxone, and aztreonam inhibited only 30.2, 9.7, and 22.4% of the isolates, respectively, under the current susceptibility breakpoint criteria (12). The activity of piperacillin-tazobactam against the isolates was also limited, and the combination was active against 59.6% of the isolates using the CLSI breakpoints (12) and 49.2% applying the EUCAST interpretative criteria (16). Meropenem inhibited 84.2 and 85.3% of the isolates under the current susceptibility breakpoint criteria for CLSI and EUCAST, respectively (12, 16). Among other antimicrobial classes, tigecycline and colistin were active against 98.8 and 89.9% of the isolates using the U.S. FDA (12) and EUCAST (16) breakpoints, respectively (Table 2).

CRE strains had elevated MIC values for most comparator agents, although ceftazidime-avibactam ($MIC_{50/90}$, 0.5/2 μg/ml; 98.5% susceptible), tigecycline ($MIC_{50/90}$, 0.5/1; 98.8% susceptible) (U.S. FDA breakpoint) and colistin ($MIC_{50/90}$, 0.5/8; 83.9% susceptible) (EUCAST breakpoint) were relatively unaffected.

Isolates harboring $bla_{CTX\text{-}M\text{-}15}$-like and/or $bla_{CTX\text{-}M\text{-}14}$-like genes without carbapenemases displayed elevated MIC values for cephalosporins (0.0 to 28.1% susceptible using CLSI criteria) (Table 2) and aztreonam (14.0% susceptible); however, ceftazidime-avibactam ($MIC_{50/90}$, 0.12/0.5 μg/ml; 100.0% susceptible) inhibited all of the isolates at the current U.S. FDA-established breakpoint. Meropenem, tigecycline, and colistin were also active against these strains (98.2, 98.9, and 94.8% susceptible, respectively).

Similar to the CRE analysis, isolates carrying bla_{KPC} were very resistant to most agents tested, including non-β-lactam agents, such as gentamicin and levofloxacin (only 48.0 and 12.5% susceptible, respectively, using the CLSI criteria) (Table 2). Ceftazidime-avibactam ($MIC_{50/90}$, 0.5/2 μg/ml) and tigecycline ($MIC_{50/90}$, 0.5/1 μg/ml) were the most active *in vitro* agents against these isolates, inhibiting 99.7 and 98.7% of the isolates at the U.S. FDA breakpoints. Colistin ($MIC_{50/90}$, 0.5/8 μg/ml) inhibited 84.1% of the isolates carrying bla_{KPC} at the EUCAST susceptible breakpoint of ≤2 μg/ml.

All but five isolates displayed ceftazidime-avibactam MIC values of ≤4 μg/ml. The five were *K. pneumoniae* isolates (ceftazidime-avibactam MIC values of >32 μg/ml) and produced metallo-β-lactamase genes. Four isolates from Colorado (two isolates), New York, and California carried $bla_{NDM\text{-}1}$, and one isolate harboring $bla_{KPC\text{-}2}$ and $bla_{VIM\text{-}4}$ was from New York.

TABLE 2 Activities of ceftazidime-avibactam and comparator antimicrobial agents tested against 2,129 ESBL phenotype-positive *Enterobacteriaceae* isolates and subgroups collected in 63 U.S. hospitals (2012 to 2014)

Organism group (no. tested) and antimicrobial agent	MIC (μg/ml)			CLSI[a] (%)			EUCAST[a] (%)		
	50%	90%	Range	S	I	R	S	I	R
ESBL phenotype (2,129)[b]									
Ceftazidime-avibactam	0.12	1	≤0.015 to >32	99.7		0.3[c]			
Ceftazidime	16	>32	0.03 to >32	30.2	10.7	59.1	13.3	16.9	69.8
Ceftriaxone	>8	>8	≤0.06 to >8	9.7	2.4	87.8	9.7	2.4	87.8
Aztreonam	>16	>16	≤0.12 to >16	22.4	7.9	69.8	11.5	10.9	77.6
Meropenem	≤0.06	>8	≤0.06 to >8	84.2	1.1	14.7	85.3	4.6	10.1
Piperacillin-tazobactam	16	>64	≤0.5 to >64	59.6	9.4	31.1	49.2	10.4	40.4
Gentamicin	2	>8	≤1 to >8	58.8	4.9	36.3	55.3	3.5	41.2
Levofloxacin	>4	>4	≤0.12 to >4	29.0	3.5	67.5	27.1	1.9	71.0
Colistin	0.5	4	0.12 to >8				89.9		10.1
Tigecycline	0.25	1	0.03 to 8	98.8	1.2	<0.1[d]	94.1	4.7	1.2
CRE (326)[e]									
Ceftazidime-avibactam	0.5	2	≤0.015 to >32	98.5		1.5[c]			
Ceftazidime	>32	>32	4 to >32	0.9	1.5	97.5	0.0	0.9	99.1
Ceftriaxone	>8	>8	4 to >8	0.0	0.0	100.0	0.0	0.0	100.0
Aztreonam	>16	>16	4 to >16	0.3	0.9	98.8	0.0	0.3	99.7
Meropenem	>8	>8	1 to >8	0.9	3.1	96.0	4.0	29.8	66.3
Piperacillin-tazobactam	>64	>64	16 to >64	0.3	1.2	98.5	0.0	0.3	99.7
Gentamicin	8	>8	≤1 to >8	48.2	13.8	38.0	38.7	9.5	51.8
Levofloxacin	>4	>4	≤0.12 to >4	13.5	1.8	84.7	11.3	2.1	86.5
Colistin	0.5	8	0.25 to >8				83.9		16.1
Tigecycline	0.5	1	0.06 to 8	98.8	0.9	0.3[d]	93.8	4.9	1.2
Isolates carrying $bla_{\text{CTX-M}}$ (1,120)[f]									
Ceftazidime-avibactam	0.12	0.5	≤0.015 to 2	100.0		0.0[c]			
Ceftazidime	16	>32	0.06 to >32	28.1	13.9	57.9	10.4	17.8	71.9
Ceftriaxone	>8	>8	4 to >8	0.0	0.0	100.0	0.0	0.0	100.0
Aztreonam	>16	>16	≤0.12 to >16	14.0	8.8	77.1	3.7	10.4	86.0
Meropenem	≤0.06	≤0.06	≤0.06 to 8	98.2	0.7	1.1	98.9	1.1	0.0
Piperacillin-tazobactam	8	64	≤0.5 to >64	77.9	12.2	9.8	62.9	15.0	22.1
Gentamicin	2	>8	≤1 to >8	52.6	1.3	46.1	51.3	1.3	47.4
Levofloxacin	>4	>4	≤0.12 to >4	13.9	4.1	82.0	12.8	1.2	86.1
Colistin	0.5	1	0.12 to >8				94.8		5.2
Tigecycline	0.12	0.5	0.03 to 4	98.9	1.1	0.0[d]	95.8	3.1	1.1
Isolates carrying bla_{KPC} (304)[g]									
Ceftazidime-avibactam	0.5	2	≤0.015 to >32	99.7		0.3[c]			
Ceftazidime	>32	>32	4 to >32	1.0	1.6	97.4	0.0	1.0	99.0
Ceftriaxone	>8	>8	8 to >8	0.0	0.0	100.0	0.0	0.0	100.0
Aztreonam	>16	>16	8 to >16	0.0	0.3	99.7	0.0	0.0	100.0
Meropenem	>64	>64	64 to >64	0.0	1.0	99.0	0.0	0.0	100.0
Piperacillin-tazobactam	>8	>8	1 to >8	1.3	4.3	94.4	5.6	26.3	68.1
Gentamicin	8	>8	≤1 to >8	48.0	14.5	37.5	38.2	9.9	52.0
Levofloxacin	>4	>4	≤0.12 to >4	12.5	1.6	85.9	9.9	2.6	87.5
Colistin	0.5	8	0.25 to >8				84.1		15.9
Tigecycline	0.5	1	0.06 to 8	98.7	1.0	0.3[d]	94.1	4.6	1.3

[a] Criteria as published by CLSI (11, 12) and EUCAST (16). S, susceptible; I, intermediate; R, resistant.
[b] ESBL phenotype isolates included *E. coli* (1,048), *K. oxytoca* (135), *K. pneumoniae* (843), and *P. mirabilis* (103).
[c] Breakpoints from U.S. FDA package insert.
[d] Breakpoints from U.S. FDA package insert (revised December 2014).
[e] CRE isolates included *E. coli* ($n = 15$), *K. oxytoca* ($n = 7$), and *K. pneumoniae* ($n = 304$).
[f] Isolates carrying $bla_{\text{CTX-M}}$ included isolates of the following species harboring $bla_{\text{CTX-M-14}}$-like and/or $bla_{\text{CTX-M-15}}$-like genes: *E. coli* ($n = 780$), *K. oxytoca* ($n = 5$), *K. pneumoniae* ($n = 290$), and *P. mirabilis* ($n = 45$).
[g] Isolates carrying bla_{KPC} included *E. coli* ($n = 11$), *K. oxytoca* ($n = 8$), and *K. pneumoniae* ($n = 285$).

DISCUSSION

Important and significant differences were detected across the 3 years of surveillance in U.S. hospitals, with the most notable changes among *E. coli* and *K. pneumoniae* isolates. *E. coli* isolates displayed an overall increase of ESBL phenotype rates over the study period but a reduced rate of isolates carrying $bla_{\text{CTX-M-15}}$-like genes that was very common among these species during the two initial years surveyed (3, 4). Conversely, the ESBL phenotype

rates decreased among *K. pneumoniae* isolates, mainly due to a reduction of isolates harboring bla_{SHV} ESBL and a decrease in CR *K. pneumoniae* and isolates carrying bla_{KPC} in 2014 compared to 2012. Furthermore, there was an increase of *K. pneumoniae* isolates carrying $bla_{CTX\text{-}M\text{-}15}$-like genes that should be closely monitored, since these isolates could be replacing the organisms carrying bla_{SHV} that usually display rates of susceptibility to other antimicrobial classes higher than those of isolates harboring $bla_{CTX\text{-}M}$ (3).

The reduced occurrences of CR *K. pneumoniae* and isolates harboring bla_{KPC} were noticed in five hospitals where the prevalence of these genes had been elevated in prior monitored study years to epidemic/endemic levels (data not shown). Nationwide CRE prevention programs carried out in Israeli hospitals reported a reduction in the CRE rates from 55.5 carriers to 11.7 carriers per 100,000 patient days in 1 year (17). The measures in Israeli prevention programs involved routine CRE screening, patient and staff cohorting with isolation of carriers and dedicated nursing staff reducing contact with noncarriers, effective communication among medical and laboratory staff, and active surveillance in acute and long-term-care facilities (17), procedures that are also recommended by the CDC (18).

Despite the recent report of bla_{KPC} and bla_{SHV} variants genetically engineered to contain mutations in important motifs that affect avibactam inhibition (19–21) and a recent report of isolates harboring bla_{KPC} with an elevated ceftazidime-avibactam MIC value (22), clinical isolates carrying bla_{KPC} and other β-lactamases collected in 63 U.S. hospitals monitored over three consecutive recent years were predominantly susceptible to ceftazidime-avibactam. The compound displayed broad activity against isolates producing the commonly observed β-lactamases that are prevalent in the United States, including isolates carrying bla_{KPC}, and is still a valuable therapy option to treat infections caused by these organisms that are usually resistant to multiple antimicrobial classes.

Avibactam is not active against metallo-β-lactamase-producing isolates, which are still very uncommon yet increasingly reported in U.S. hospitals. Aztreonam-avibactam has demonstrated activity against these isolates (23–26), since the metalloenzymes do not hydrolyze monobactams and avibactam would inhibit other β-lactamases present in the isolate; however, further *in vivo* and *in vitro* investigations of this new combination are warranted.

In summary, a robust 3-year analysis of the frequencies of ESBL- and carbapenemase-producing *Enterobacteriaceae* isolates demonstrated that important changes are occurring in U.S. medical centers.

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