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MAJOR ARTICLE

Trends of β-Lactamase Occurrence Among *Escherichia coli* and *Klebsiella pneumoniae* in United States Hospitals During a 5-Year Period and Activity of Antimicrobial Agents Against Isolates Stratified by β-Lactamase Type

Mariana Castanheira, John H. Kimbrough, Sean DeVries, Rodrigo E. Mendes, and Helio S. Sader

JMI Laboratories, North Liberty, Iowa, USA

Background. The temporal and longitudinal trends of β-lactamases and their associated susceptibility patterns were analyzed for *Escherichia coli* and *Klebsiella pneumoniae* isolates consecutively collected in 56 United States hospitals during 2016–2020.

Methods. Isolates (n = 19 453) were susceptibility tested by reference broth microdilution methods. Isolates that displayed minimum inhibitory concentration (MIC) values ≥2 mg/L for at least 2 of the following compounds—ceftazidime, ceftriaxone, aztreonam, or cefepime—or resistance to the carbapenems were submitted to whole genome sequencing for identification of β-lactamases. Longitudinal and temporal trends were determined by slope coefficient. New CTX-M and OXA-1 variants were characterized.

Results. Extended-spectrum β-lactamases (ESBLs) were detected among 88.0% of the isolates that displayed elevated cephalosporin/aztreonam MICs without carbapenem resistance. $bla_{\text{CTX-M-15}}$ was detected among 55.5% of the ESBL producers. ESBL rates were stable over time, but significant increases were noted among bloodstream infection and *K pneumoniae* isolates, mainly driven by an increase in $bla_{\text{CTX-M}}$. Carbapenem resistance and carbapenemase genes were noted among 166 and 145 isolates, respectively, including 137 bla_{KPC}, 6 bla_{SME}, 3 $bla_{\text{OXA-48}}$–like, and 3 bla_{NDM}. Ceftazidime-avibactam and carbapenems were very active (>99% susceptibility) against ESBL producers without carbapenem resistance. Ceftazidime-avibactam inhibited 97.0% of the carbapenem-resistant isolates. This agent and meropenem-vaborbactam inhibited 96.4% and 85.0% of the 2020 isolates, respectively.

Conclusions. Overall, ESBL-producing isolates were stable, but an increase was noted for *K pneumoniae* isolates driven by CTX-M production. Carbapenem-resistant Enterobacterales rates decreased in the study period. The prevalence of metallo-β-lactamases and OXA-48–like remains low. Continuous surveillance of β-lactamase–producing isolates is prudent.

Keywords. β-lactamases; carbapenemase; ESBL; trends; United States.

Due to their desirable safety profile and broad spectrum of activity against gram-positive and gram-negative pathogens, β-lactam agents are the most used antimicrobial class for the treatment of bacterial infections [1]. Lamentably, many bacterial isolates carry resistance mechanisms that limit the use of these agents. Of these mechanisms, β-lactamase production is the most common among Enterobacterales spp [2]. These enzymes include extended-spectrum β-lactamases (ESBLs), transferrable cephalosporinases or AmpCs, and carbapenemases exhibiting diverse substrate profiles, which leads to the inactivation of penicillins, cephalosporins, and/or carbapenems [3]. Most β-lactamase genes are carried in plasmids or other mobile elements that harbor additional resistance mechanisms. Moreover, point mutations can expand their hydrolytic spectra [2]. Understanding the epidemiology of isolates producing β-lactamases is important to direct appropriate antimicrobial chemotherapy locally and to inform further development of compounds in the antimicrobial pipeline that would have efficacy against these isolates globally [4, 5].

There are numerous studies evaluating the prevalence of ESBLs, transferable AmpCs, and carbapenemases in United States (US) hospitals; however, due to differences in methodology and patient populations, aggregating these studies to provide a national scenario is challenging [6, 7]. Jernigan et al [8] analyzed a cohort of patients hospitalized in the US and concluded that 32% of the infections were caused by ESBL-producing isolates. The same authors observed that the incidence of carbapenem-resistant Enterobacterales was stable in the study period and ranged from 3.36 to 3.79 cases per 10 000 hospitalizations. No data regarding the type of ESBL

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Correspondence: Mariana Castanheira, PhD, JMI Laboratories, 345 Beaver Kreek Center, Suite A, North Liberty, IA 52317 (mariana-castanheira@jmilabs.com); Helio S. Sader, MD, PhD, JMI Laboratories, 345 Beaver Kreek Center, Suite A, North Liberty, IA 52317 (helio-sader@jmilabs.com).

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or mechanism of carbapenem resistance were provided. In a literature review, McDanel et al concluded that the incidence of infections caused by ESBL-producing *Escherichia coli* and *Klebsiella* spp in the US increased from 1997 to 2011 [9]. This finding is corroborated by an analysis of 20 years of the SENTRY Antimicrobial Surveillance Program, which evaluated >60 000 Enterobacterales isolates collected from 199 US hospitals [10]. The ESBL phenotype rates [11] among *E coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Proteus mirabilis* increased from 4.8% in 1997–2000 to 15.7% in 2013–2016. In a study evaluating the genetic characterization of β-lactamases in US hospitals from 2012 to 2014, ESBL phenotype rates increased in *E coli* (12.7% to 15.1%) but decreased for *K pneumoniae* (18.9% to 15.5%) [12]. When analyzing the occurrence of β-lactamase genes, an increase of CTX-M-15–producing *E coli* and *K pneumoniae* was accompanied by a decrease of SHV ESBL and *K pneumoniae* carbapenemase (KPC) enzymes in *K pneumoniae*. Unfortunately, none of these reports provide a comprehensive and/or current representation of the prevalence of β-lactamases in US hospitals.

In this study, we evaluated the β-lactamase production among *E coli* and *K pneumoniae* isolates collected in 56 US hospitals between 2016 and 2020. Isolates were susceptibility tested by the reference broth microdilution method. Isolates displaying elevated minimum inhibitory concentration (MIC) values against third- and/or fourth-generation cephalosporins, aztreonam, and the carbapenems were screened for the presence of β-lactamases using whole genome sequencing (WGS). Additionally, we evaluated the susceptibility of isolates carrying common β-lactamases against clinically available antimicrobial agents.

METHODS

Bacterial Isolates

A total of 19 453 *E coli* and *K pneumoniae* isolates were collected in 56 US hospitals from 2016 to 2020 as part of the International Network for Optimal Resistance Monitoring (INFORM) Surveillance Program [13]. Only participating hospitals that submitted isolates each year of the study period were included. Bacterial isolates were deemed to be the cause of infection by local clinical and/or microbiological criteria were included in this investigation. Participating sites were asked to submit consecutively collected isolates from patients hospitalized with pneumonia (PHP; n = 2643) as well as urinary tract (UTI; n = 9999), bloodstream (BSI; n = 4152), skin and skin structure (SSSI; n = 1407), and intra-abdominal (IAI; n = 1252) infections.

Species identification was confirmed when needed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) using the Bruker Daltonics MALDI Biotyper (Billerica, Massachusetts) following the manufacturer's instructions. All isolates submitted to genomic analysis were submitted to MALDI-TOF MS identification.

Susceptibility Testing

Antimicrobial susceptibility testing was performed by reference broth microdilution methods conducted according to Clinical and Laboratory Standards Institute (CLSI) procedures [14]. Quality control testing was performed daily to ensure proper test conditions. Quality control strains included *E coli* ATCC 25922 and NCTC 13353, *K pneumoniae* ATCC 700603, ATCC BAA-1705 and BAA-2814, and *Pseudomonas aeruginosa* ATCC 27853. CLSI guidelines were used for the interpretation of susceptibility rates, with the exception of tigecycline, for which US Food and Drug Administration (FDA) breakpoints were applied [15, 16]. Avibactam was provided by Allergan. Other agents were acquired from Sigma-Aldrich (St Louis, Missouri), US Pharmacopeia (Rockville, Maryland), Advanced Chemblocks (Hayward, California; relebactam), or MedChemExpress (Monmouth Junction, New Jersey; vaborbactam).

β-Lactamase Screening

All isolates displaying MIC values ≥2 mg/L for at least 2 of the following β-lactams—ceftazidime, ceftriaxone, aztreonam, or cefepime—and/or displaying meropenem and/or imipenem MIC results ≥2 mg/L were submitted to WGS. Total genomic DNA was prepared using the Nextera XT library construction protocol and index kit (Illumina, San Diego, California) following the manufacturer's instructions and then sequenced on a MiSeq Sequencer (Illumina) with a target coverage of 30X. FASTQ format files for each sample set were assembled independently using de novo assembler SPAdes 3.9.0 [17] with K-values of 21, 33, 55, 77, and 99 and careful mode on to reduce the number of mismatches. This process produced a FASTA format file of contiguous sequences with the best N50 value. An in house–designed software using the target assembled sequences [18] as queries to align against numerous resistance determinants from the National Center for Biotechnology Information Bacterial Antimicrobial Resistance Reference Gene Database (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA313047) was used to search for β-lactamase genes. Potential matches were generated with the criteria of >94% identity and 40% minimum coverage length [19].

Characterization of β-Lactamase–Encoding Genes

The activities of uncharacterized CTX-M- and OXA-type β-lactamases were determined by cloning the upstream region, open reading for target alleles, and subsequently expressing each target allele in *E coli* DH5α [20] to determine MIC values against a panel of β-lactams and carbapenems. Oligonucleotides were synthesized by Integrated DNA Technologies (Coralville, Iowa). Q5 2X polymerase chain reaction (PCR) master mix and NEB 5-alpha (C2987H)–competent cells were obtained from New England Biolabs (Waltham, Massachusetts). PCR clean-ups were performed using the DNA Clean and Concentrator-5 kit (Zymo Research, Irvine, California). In brief, primer pairs unique

to the CTX-M group 1 (CTXM_G1F 5′AGCAGTCTAAATTC TTCGTGAAATAGTG3′/CTX-M-15-cloning-R 5′TTACAAAC CGTCGGTGACGATTT 3′), CTX-M group 9 (CTXM_G9F 5′ GTTACAATGTGTGAGAAGCAGT3′/CTX Gr9 S-R 5′TTACA GCCCTTCGGCGAT3′), or OXA-1_OXA-30 (OXA1cloneNewF 5′AACACGCAGTGATGCCTAA3′/OXA1cloneNewR 5′TTAT AAATTTAGTGTGTTTAGAATGGTGATC3′) were used to generate PCR products that were blunt-end cloned into pCR-Blunt II using the Zero Blunt TOPO PCR cloning kit (Invitrogen, Carlsbad, California), transformed into *E coli* DH5α, and selected on Luria Bertani agar plates containing 50 mg/L of kanamycin. The presence and orientation of inserts was confirmed by PCR and sequenced. Constructs were then introduced into DH5α via electroporation and MIC testing was performed as described above.

Statistical Analysis

The data were analyzed using R Studio version 2022.02.00. The *P* values in the statistically significant column in Figure 1 were found using a linear regression model for the percentages from year to year. A significant finding is when the coefficient for change in percentage by year is statistically different than zero. This coefficient can be seen as the average change in percentage from year to year; therefore, when the number is statistically different than zero, there is a clear linear change over the 5 years included.

RESULTS

β-Lactamase Production Among Carbapenem-Susceptible or -Intermediate Isolates

Among 3026 of 19 453 (15.6%) *E coli* and *K pneumoniae* isolates that were screened for β-lactamase production due to increased MIC values for 2 cephalosporins and/or aztreonam without carbapenem resistance, 2277 were *E coli* and 749 were *K pneumoniae* (Tables 1 and 2). These numbers correspond to 16.3% and 13.7% of all isolates from these species, respectively.

Overall, ESBL production was noted among 14.2% (1977/13 968) of the *E coli* isolates and 12.5% (686/5485) of the *K pneumoniae* isolates (Tables 1 and 2). These rates corresponded to 86.8% and 91.6% of the isolates resistant to cephalosporins and/or aztreonam without carbapenem resistance, respectively. ESBL production varied across US census divisions. For both species, ESBL production was highest in the Middle Atlantic and corresponded to 29.5% of the *E coli* and 19.3% of the *K pneumoniae* isolates from this census division. The lowest ESBL production rate among *E coli* isolates was noted in the West North Central division (6.2%). *Escherichia coli* ESBL production rates in the remaining census divisions ranged from 14.8% to 16.3% in East South Central, West South Central, and Pacific and 9.3% to 10.4% in East North Central, South Atlantic, Mountain, and New England. ESBL production rates in *K pneumoniae* isolates collected in the West South Central division were 16.7% and ranged from 10.2% to 10.4% in the New England, South Atlantic, and Pacific divisions. These rates were lower in the 4 remaining census divisions and ranged from 8.1% to 8.9%.

Of all β-lactamase genes observed, $bla_{\text{CTX-M-15}}$ was the most common and was detected among 1771 of the 3026 (55.5%) screened isolates, including 1193 (52.4%) of the *E coli* and 533 (71.2%) of the *K pneumoniae* isolates. The genes encoding CTX-M-55, CTX-M-14, and CTX-M-27 were common among *E coli* isolates and were detected in 104 (4.5%), 167 (7.4%), and 426 (18.5%) of these isolates, respectively. Only 25 *K pneumoniae* isolates carried these genes. Twenty less common CTX-M variants were detected among 92 isolates that were mostly *E coli* (65 isolates).

SHV enzymes with an ESBL spectrum were detected among 188 isolates including 171 *K pneumoniae* (19.1% of the isolates tested for this species). $bla_{\text{SHV-12}}$ was detected among 86 isolates overall, followed by $bla_{\text{SHV-7}}$ and $bla_{\text{SHV-27}}$ that were detected among 32 and 23 isolates, respectively. Genes encoding TEM ESBL enzymes were detected among only 16 isolates (11 *E coli* and 5 *K pneumoniae*).

Among transferable class C enzymes, CMY-2 was observed among 179 *E coli* isolates and only 10 *K pneumoniae*. DHA-1 was detected among 36 isolates (19 *E coli* and 17 *K pneumoniae*) and FOX-5 was detected among 8 isolates, mostly *K pneumoniae*. Despite not being a true ESBL due to the lack of inhibition by older inhibitors, such as clavulanate and tazobactam, OXA-1 or OXA-1–like was detected in 1121 isolates and was mostly associated with CTX-M-15 (96.8%, n = 1085).

Six novel or uncharacterized $bla_{\text{CTX-M}}$ and 1 novel $bla_{\text{OXA-1}}$ were isolated during the surveillance period. Each variant and their closest characterized relatives were cloned and expressed in the β-lactam–susceptible *E coli* DH5α. All CTX-M-15 variants (A15T, A112T, and V234A) displayed increased activity against all β-lactam agents, except for meropenem, relative to its ancestral CTX-M-15 (Table 3). *Escherichia coli* DH5α harboring any of the other 3 new variants, CTX-M-14$^{\text{V151M}}$, CTX-M-27$^{\text{G239S}}$, and CTX-M-55$^{\text{G239S}}$, displayed higher susceptibility to all tested β-lactam agents relative to strains expressing the wild-type alleles. Interestingly, strains expressing CTX-M-27$^{\text{G239S}}$ or CTX-M-55$^{\text{G239S}}$ essentially mirrored the susceptibility pattern of DH5α, although resistance to piperacillin was elevated. CTX-M-27 and CTX-M-55 belong to the divergent lineages of group 9 and group 1 within the CTX-M phylogeny, and it is intriguing that a shared mutation near several highly conserved and well-characterized residues involved in the activity of these enzymes essentially eliminates functionality against most targets.

The OXA-1$^{\text{S209N}}$ harboring strain was less susceptible to piperacillin and cefepime (2- and 4-fold increases in MIC, respectively), but was more susceptible to aztreonam and ceftazidime (2- and 4-fold decrease in MIC, respectively; Table 3).

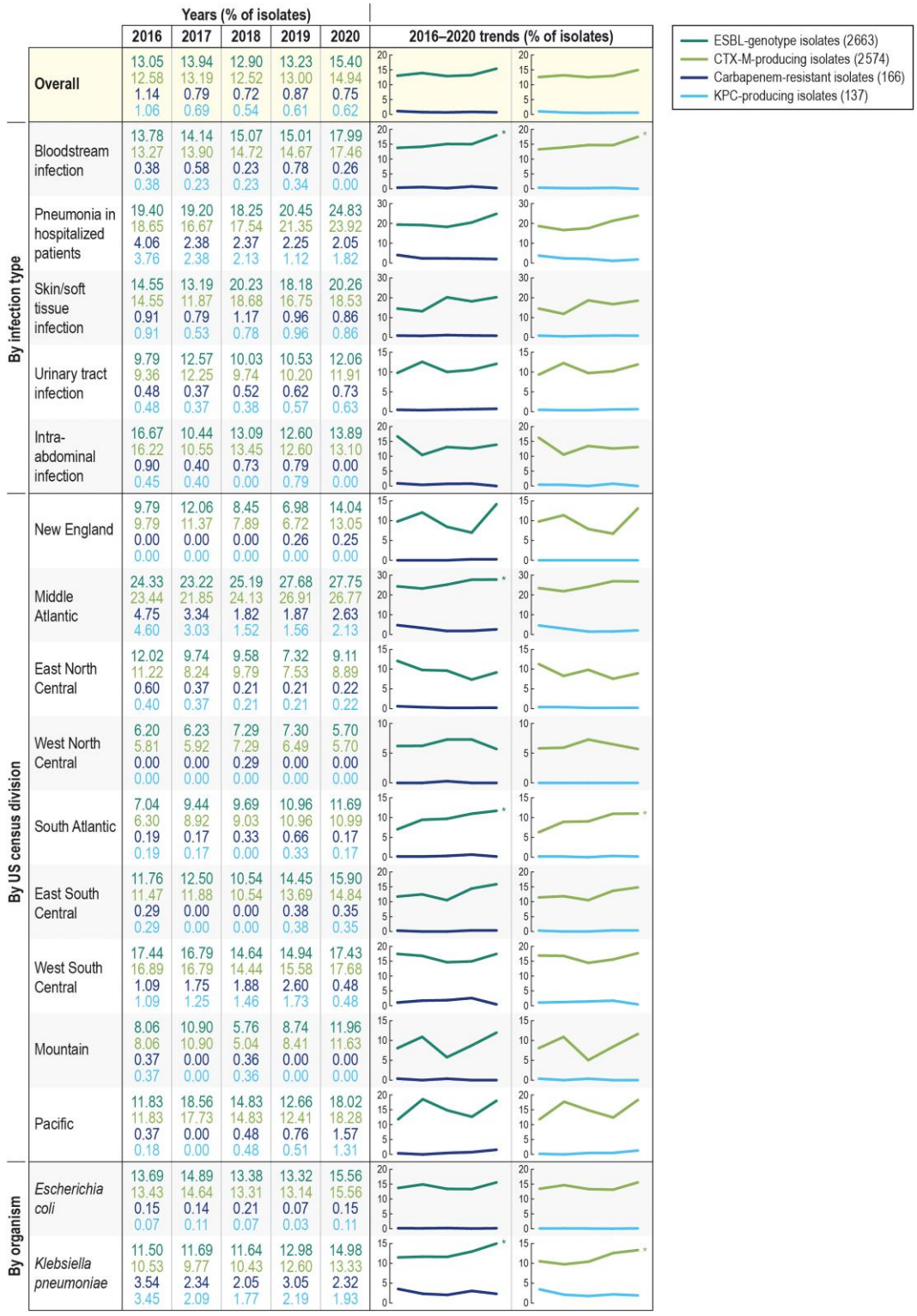

Figure 1. Trend analysis of extended-spectrum β-lactamase (ESBL)–producing, CTX-M–producing, carbapenem-resistant, and *Klebsiella pneumoniae* carbapenem (KPC)–producing isolates collected during 2016–2020. *Statistically significant.

The distribution of CTX-M–producing isolates was very similar among all US census divisions, ranging from 78.2% to 84.5%. No striking geographic differences in the occurrence of distinct variants was noted (data not shown). A similar scenario was noted with SHV ESBLs and CMY enzymes. Other less common ESBLs had more variability among census divisions.

Table 1. Results for β-Lactamase Screening for 2297 *Escherichia coli* Isolates From US Hospitals (2016–2020) Displaying Elevated Cephalosporins or Aztreonam Minimum Inhibitory Concentration Values

	No. of Positive Results (% of Isolates Over the Tested)									
β–Lactamase	National	New England	Middle Atlantic	East North Central	West North Central	South Atlantic	East South Central	West South Central	Mountain	Pacific
Carbapenem susceptible and intermediate										
No. tested	2277/13968 (16.3)	186/1494 (12.4)	652/1970 (33.1)	211/1813 (11.6)	94/1231 (7.6)	232/2001 (11.6)	172/1028 (16.7)	270/1537 (17.6)	130/1116 (11.6)	330/1778 (18.6)
ESBLs										
CTX-M	1944 (85.4)	152 (81.7)	576 (88.3)	173 (82.0)	75 (79.8)	188 (81.0)	150 (87.2)	240 (88.9)	103 (79.2)	287 (87.0)
CTX-M-1 group[a]	1329 (58.4)	97 (52.2)	366 (56.1)	128 (60.7)	52 (55.3)	116 (50.0)	96 (55.8)	179 (66.3)	78 (60.0)	217 (65.8)
CTX-M-2 group[b]	4 (0.2)	0	4 (0.6)	0	0	0	0	0	0	0
CTX-M-8 + CTX-M-25 group[c]	2 (0.1)	0	0 (0.0)	1 (0.5)	0	0	0	0	0	1 (0.3)
CTX-M-9 group[d]	619 (27.2)	55 (29.6)	209 (32.1)	45 (21.3)	23 (24.5)	74 (31.9)	54 (31.4)	64 (23.7)	25 (19.2)	70 (21.2)
OXA-1–like	720 (31.6)	40 (21.5)	214 (32.8)	67 (31.8)	21 (22.3)	69 (29.7)	42 (24.4)	108 (40.0)	44 (33.8)	115 (34.8)
SHV[e]	16 (0.7)	2 (1.1)	7 (1.1)	3 (1.4)	1 (1.1)	0	2 (1.2)	0	0	1 (0.3)
TEM[f]	11 (0.5)	1 (0.5)	1 (0.2)	0	0	2 (0.9)	0	5 (1.9)	1 (0.8)	1 (0.3)
Transferable AmpC[g]	226 (9.9)	24 (12.9)	50 (7.7)	26 (12.3)	11 (11.7)	30 (12.9)	13 (7.6)	21 (7.8)	18 (13.8)	33 (10.0)
Carbapenemases										
KPC-2	2 (0.1)	0	1 (0.2)	0	0	0	0	1 (0.4)	0	0
KPC-3	5 (0.2)	1 (0.5)	4 (0.3)	0	0	0	0	0	0	0
KPC-4	1 (<0.1)	0	0	0	0	0	0	0	1 (0.8)	0
NDM-5–like	1 (<0.1)	0	0	0	0	0	0	1 (0.4)	0	0
OXA-181	3 (0.1)	0	1 (0.2)	2 (0.9)	0	0	0	0	0	0
Carbapenem resistant										
No. tested	20/13968 (0.1)	0/1494 (0.0)	5/1970 (0.3)	2/1813 (<0.1)	1/1231 (<0.1)	1/2001 (<0.1)	0/1028 (0.0)	7/1537 (0.4)	0/1116 (0.0)	4/1778 (0.2)
Carbapenemases										
KPC-2	8 (40.0)	0	2 (40.0)	1 (50.0)	0	0	0	3 (42.9)	0	2 (50.0)
KPC-3	3 (15.0)	0	2 (40.0)	0	0	0	0	0	0	1 (25.0)
NDM-1	1 (5.0)	0	0	0	0	0	0	1 (14.3)	0	0
NDM-5	1 (5.0)	0	1 (20.0)	0	0	0	0	0	0	0
Carbapenemase negative	7 (35.0)	0	0	1 (50.0)	1 (100)	1 (100.0)	0	3 (42.9)	0	1 (25.0)

Abbreviation: ESBL, extended-spectrum β-lactamase.

[a]One hundred ninety-three CTX-M-15, 104 CTX-M-55, 14 CTX-M-3, 8 CTX-M-1, 6 CTX-M-15–like, 1 CTX-M-10, 1, 1 CTX-M-32, 1 CTX-M-55–like, 1 CTX-M-64.

[b]Four CTX-M-115.

[c]One CTX-M-8, 1 CTX-M-100.

[d]Four hundred twenty-six CTX-M-27, 167 CTX-M-14, 8 CTX-M-65, 8 CTX-M-134, 5 CTX-M-24, 2 CTX-M-27–like, 1 CTX-M-9, 1 CTX-M-19, 1 CTX-M-174.

[e]Eleven SHV-12, 2 SHV-12–like, 1 SHV-5, 1 SHV-7, 1 SHV-18–like.

[f]Four TEM-19, 2 TEM-12, 2 TEM-169, 1 TEM-29–like, 1 TEM-52, 1 TEM-123.

[g]One hundred seventy-nine CMY-2, 19 DHA-1, 11 CMY-42, 4 CMY-4, 3 CMY-2–like, 2 CMY-4–like, 2 FOX-5, 1 CMY-16–like, 1 CMY-42–like, 1 CMY-44, 1 CMY-44–like, 1 CMY-102–like, 1 CMY-11.

Table 2. Results for β-Lactamase Screening for 897 *Klebsiella pneumoniae* Isolates From US Hospitals (2016–2020) Displaying Elevated Cephalosporins or Aztreonam Minimum Inhibitory Concentration Values

	No. of Positive Results (% of Isolates Over the Tested)									
β–Lactamase	National	New England	Middle Atlantic	East North Central	West North Central	South Atlantic	East South Central	West South Central	Mountain	Pacific
Carbapenem susceptible and intermediate										
No. tested	751/5485 (13.7)	51/463 (11.0)	273/1274 (21.4)	61/639 (9.5)	30/359 (8.4)	101/895 (11.3)	45/472 (9.5)	106/582 (18.2)	35/357 (9.8)	49/444 (11.0)
ESBLs										
CTX-M	579 (77.3)	40 (80.0)	203 (74.4)	48 (78.7)	24 (80.0)	75 (75.0)	36 (80.0)	85 (80.2)	28 (80.0)	40 (81.6)
CTX-M-1 group[a]	554 (74.0)	36 (72.0)	197 (72.2)	48 (78.7)	22 (73.3)	69 (69.0)	35 (77.8)	83 (78.3)	26 (74.3)	38 (77.6)
CTX-M-2 group[b]	1 (0.1)	0	0	0	0	1 (1.0)	0	0	0	0
CTX-M-8 + CTX-M-25 group[c]	1 (0.1)	0	1 (0.4)	0	0	0	0	0	0	0
CTX-M-9 group[d]	26 (3.5)	4 (8.0)	6 (2.2)	0 (0.0)	2 (6.7)	5 (5.0)	1 (2.2)	3 (2.8)	3 (8.6)	2 (4.1)
OXA-1–like	368 (49.1)	22 (44.0)	127 (46.5)	30 (49.2)	11 (36.7)	49 (49.0)	27 (60.0)	62 (58.5)	13 (37.1)	27 (55.1)
SHV[e]	135 (18.0)	8 (16.0)	49 (17.9)	8 (13.1)	6 (20.0)	21 (21.0)	10 (22.2)	22 (20.7)	4 (11.4)	7 (14.3)
TEM[f]	4 (0.5)	0	2 (0.7)	2 (3.3)	0	0	0	0	0	0
Transferable AmpC[g]	31 (4.1)	1 (2.0)	12 (4.4)	3 (4.9)	1 (3.3)	4 (4.0)	1 (2.2)	6 (5.7)	1 (2.9)	2 (4.1)
Carbapenemases										
KPC-2	1 (0.1)	0	0	0	0	0	0	1 (0.9)	0	0
KPC-3	1 (0.1)	0	1 (0.4)	0	0	0	0	0	0	0
OXA-181	1 (0.1)	0	0	0	0	0	0	1 (0.9)	0	0
OXA-232	1 (0.1)	0	0	1 (1.6)	0	0	0	0	0	0
OXA-48	1 (0.1)	0	0	0	0	1 (1.0)	0	0	0	0
Carbapenem resistant										
No. tested	146/5485 (2.7)	2/463 (0.4)	89/1274 (7.0)	6/639 (0.9)	0/359 (0.0)	8/895 (0.9)	3/472 (0.6)	27/582 (4.6)	2/357 (0.6)	9/444 (2.0)
Carbapenemases										
KPC-2	58 (1.1)	0	32 (2.5)	1 (0.2)	0	3 (0.3)	1 (0.2)	19 (3.3)	1 (0.3)	1 (0.2)
KPC-2–like	2 (<0.1)	0	0	0	0	0	1 (0.2)	1 (0.2)	0	0
KPC-3	66 (1.2)	0	48 (3.8)	5 (0.8)	0	2 (0.2)	1 (0.2)	3 (0.5)	1 (0.3)	6 (1.4)
NDM-1, OXA-232	1 (<0.1)	0	1 (0.1)	0	0	0	0	0	0	0
NDM-5	2 (<0.1)	1 (0.2)	1 (0.1)	0	0	0	0	0	0	0
OXA-232	2 (<0.1)	0	2 (0.2)	0	0	0	0	0	0	0
OXA-48	1 (<0.1)	0	0	0	0	1 (0.1)	0	0	0	0
Carbapenemase negative	14 (0.3)	1 (0.2)	5 (0.4)	0	0	2 (0.2)	0	4 (0.7)	0	2 (0.5)

Abbreviation: ESBL, extended-spectrum β-lactamase.

[a]Five hundred thirty-three CTX-M-15, 16 CTX-M-3, 2 CTX-M-1, 2 CTX-M-55, 1 CTX-M-15–like.

[b]One CTX-M-2.

[c]One CTX-M-8.

[d]Eighteen CTX-M-14, 5 CTX-M-27, 2 CTX-M-65, 1 CTX-M-14–like.

[e]Forty-three SHV-12, 71 SHV-106, 30 SHV-7, 21 SHV-2, 12 SHV-2, 6 SHV-5, 4 SHV-30, 3 SHV-2–like, 3 SHV-5–like, 2 SHV-18, 2 SHV-40, 1 SHV-12–like, 1 SHV-154, 1 SHV-154–like, 1 SHV-2A, 1 SHV-38, 1 SHV-42, 1 SHV-55, 1 SHV-9.

[f]One TEM-12–like, 1 TEM-16, 1 TEM-26, 1 TEM-29.

[g]Seventeen DHA-1, 9 CMY-2, 3 FOX-5, 1 CMY-4, 1 FOX-5–like.

Table 3. Susceptibility Profiles of Uncharacterized and Novel CTX-M- and OXA-Type Enzymes Identified in This Study

Isolate and Amino Acid Substitutions	MIC Values, mg/L					
	Ceftriaxone	Ceftazidime	Aztreonam	Meropenem	Cefepime	Piperacillin
Escherichia coli DH5α	0.12	0.25	0.12	0.12	0.06	1
E coli DH5α expressing						
CTX-M-15	64	16	32	0.12	4	128
A15T (CTX-M-232)	>256	>256	>256	0.12	32	>512
A112T (CTX-M-193)	256	256	>256	0.12	32	512
V234A	128	64	64	0.12	16	256
CTX-M-55	256	256	256	0.12	32	>512
G239S	0.12	0.25	0.12	0.12	0.06	128
CTX-M-14	64	4	32	0.12	8	256
V151M	8	2	2	0.12	4	64
CTX-M-27	64	16	32	0.12	8	256
G239S	0.12	0.12	0.25	0.12	0.06	128
OXA-1_OXA-30	0.12	0.5	0.25	0.12	0.12	16
S209N	0.12	0.12	0.12	0.12	0.5	32

Abbreviation: MIC, minimum inhibitory concentration.

A total of 11 isolates that did not display carbapenem resistance carried carbapenemase genes, including 4 $bla_{\text{OXA-181}}$ and 1 each of $bla_{\text{OXA-48}}$, $bla_{\text{OXA-232}}$, $bla_{\text{KPC-3}}$, and $bla_{\text{KPC-2}}$. Most of these isolates (5/8) were *E coli* that displayed a modal imipenem MIC value of 2 mg/L (MIC range, 1–2 mg/L) and MIC values ranging from 1 to 2 mg/L.

Most isolates had a combination of β-lactamases, including other enzymes with narrow or undermined spectrum of activity. The most common combinations in *E coli* and *K pneumoniae* included CTX-M-15, OXA-1 with or without TEM-1, and/or SHV-1–like (SHV-1, SHV-11, SHV-28, or SHV-168), which corresponded to 1509 (48.4%) isolates screened from these species.

β-Lactamase Production Among Carbapenem-Resistant Isolates

A total of 166 *E coli* and *K pneumoniae* isolates displayed carbapenem resistance (resistance to imipenem and/or meropenem), and carbapenemases were detected among 145 of these isolates (Table 1). Most carbapenem-resistant isolates were *K pneumoniae* (146/166). Carbapenemase-producing isolates included 69 isolates producing KPC-3, 66 producing KPC-2, and 1 each producing the recently described KPC-58 and KPC-59 [21]. Metallo-β-lactamases (MBLs) and OXA-48–like enzymes are still uncommon both in US hospitals and in this 5-year survey. Only 5 isolates produced MBLs, including 2 *E coli*, each carrying $bla_{\text{NDM-1}}$ or $bla_{\text{NDM-5}}$, 2 *K pneumoniae* harboring $bla_{\text{NDM-5}}$, and 1 *K pneumoniae* carrying $bla_{\text{NDM-1}}$ plus $bla_{\text{OXA-232}}$. Additionally, 3 *K pneumoniae* isolates harbored either $bla_{\text{OXA-232}}$ or $bla_{\text{OXA-48}}$.

Most of the 166 carbapenem-resistant and 145 carbapenemase-producing *E coli* and *K pneumoniae* isolates were concentrated in the Middle Atlantic census division; 94 *E coli* and 89 *K pneumoniae* isolates were collected from this division. After the Middle Atlantic division, carbapenem-resistant isolates were most often noted in the West South Central division (34 isolates) but less often isolated from the Pacific (13 isolates), South Atlantic (9 isolates), and East North Central (8 isolates) divisions. Only 1 to 3 carbapenem-resistant isolates were noted in the East South Central, Mountain, New England, and West North Central divisions.

Among the 21 carbapenem-resistant isolates that were carbapenemase negative, 16 harbored $bla_{\text{CTX-M-15}}$ with or without OXA-1 and other limited-spectrum enzymes. The remaining isolates carried a $bla_{\text{CTX-M-15}}$ variant, $bla_{\text{CMY-2}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-71}}$, or $bla_{\text{SHV-12}}$.

Trends in Occurrence of β-Lactamases

Overall, the occurrence of ESBL-producing *E coli* and *K pneumoniae* isolates displayed small variations during the study period (13.1% in 2016 to 15.4% in 2020; Figure 1), but greater variations were noted among isolates from different infection types over time. A significant increase in ESBL-producing isolates from BSI (4.2%; slope coefficient, 0.9; $P = .045$) was observed during the study period. ESBL-producing isolates also increased among PHP and SSSI: there was a 5.4% and 5.7% increase from 2016 to 2020, respectively. ESBL production slightly decreased among IAI isolates (−2.8% from 2016 to 2020).

A statistically significant increase in ESBL-producing isolates was noted among isolates from the Middle Atlantic (slope coefficient, 1.1; $P = .05$) and the South Atlantic (slope coefficient, 1.1; $P = .01$) census divisions. Additionally, an increase of 3.9% to 6.2% in ESBL-producing isolates was noted from 2016 to 2020 in the New England, East South Central, Mountain, and Pacific census divisions. When this analysis was performed by organism, ESBL production significantly increased among *K pneumoniae* (3.5%; slope coefficient, 0.8; $P = .05$).

Similar to the increase in ESBL-producing isolates, CTX-M–producers increased among isolates from BSI (4.2%; slope coefficient, 0.9; $P = .03$), PHP (5.3%), SSSI (4.0%), and the South Atlantic census division (4.7%; slope coefficient, 1.1; $P = .02$), but decreased among IAI (−3.1%) and East North Central (−2.3%) census division isolates. Notably, when these trend analyses were performed by organism, ESBL and CTX-M production significantly increased among *K pneumoniae* (3.5% and 2.8%; slope coefficient, 0.8; $P = .05$ for both) when compared to *E coli*, which displayed similar rates in the study period.

Carbapenem-resistant enterobacterales (CRE) isolates progressively declined from 44 isolates in 2016 to 28 isolates in 2020. This decline was observed mainly among PHP (27 vs 9 isolates; −2.0%) and in the Middle Atlantic census division (32 vs 16 isolates; −2.1%). When analyzing the types of carbapenemases, the number of KPC-3–producing isolates declined from 22 in 2016 to 13 in 2020. Like the overall CRE isolates, this decline was noted among isolates from PHP and the Middle Atlantic census division (data not shown). As many isolates producing KPC-3 were *K pneumoniae* (67/73), the decline in CRE isolates was associated with that species (22 vs 13 isolates from 2016 to 2020).

Susceptibility Profiles of β-Lactamase–Producing Isolates

Ceftazidime-avibactam, meropenem, and imipenem were the most active β-lactam agents tested against 2663 ESBL-producing *E coli* and *K pneumoniae* isolates that were carbapenem susceptible (Figure 2). These agents inhibited >99.9%, 99.5%, and 99.7% of the isolates, respectively. Meropenem-vaborbactam was tested only against 2020 isolates (n = 464), and this agent inhibited all the isolates at the CLSI breakpoint. Ceftolozane-tazobactam was not tested during 2016, but overall, this agent inhibited 92.1% of the ESBL-producing *E coli* and *K pneumoniae* from isolates collected from 2017 to 2022. The activity of this combination slightly decreased against *K pneumoniae* isolates when compared to *E coli* isolates (84.1% vs 94.9%). Piperacillin-tazobactam inhibited only 50.4% of the ESBL producers, including 71.4% of the 2523 CTX-M producers and 56.5% of the SHV ESBL producers (data not shown). Among other classes, tigecycline and amikacin inhibited 99.7% and 94.5% of the isolates, and 97.1% of the isolates displayed a colistin intermediate MIC value. Levofloxacin inhibited only 20.0% of the ESBL-producing isolates, and this agent exhibited lower activity against *E coli* isolates when compared to *K pneumoniae* (13.7% vs 38.3%).

Against 256 isolates harboring transferrable AmpC genes, meropenem inhibited all isolates at the current CLSI breakpoints. Ceftazidime-avibactam and imipenem were active against 99.6% and 98.4% of these isolates while ceftolozane-tazobactam inhibited 79.8% of the isolates from 2017 to 2020 (n = 208; Figure 2). Meropenem-vaborbactam inhibited all 45 isolates from 2020. Cefepime and piperacillin-tazobactam inhibited 82.4% and 66.1% of the isolates, respectively. Amikacin and tigecycline displayed similar activity, inhibiting 98.8% of the isolates producing acquired AmpCs.

Ceftazidime-avibactam was the most active agent against CRE isolates, inhibiting 97.0% of these isolates according to CLSI breakpoints (Figure 3). Meropenem-vaborbactam inhibited 85.0% of the 20 CRE isolates from 2020. All other β-lactams had limited activity against these isolates. Tigecycline was the most active non–β-lactam agent and inhibited 96.4% of the isolates applying the US FDA breakpoint. Amikacin and gentamicin inhibited 72.9% and 52.4% of the isolates, respectively, and 90.2% of the CRE isolates had an intermediate colistin MIC value.

Among 141 carbapenem-resistant isolates producing serine carbapenemases, including KPC, SME, and OXA-48–like genes, 139 (98.6%) were susceptible to ceftazidime-avibactam (Figure 3). From the 2 isolates resistant to this combination, 1 carried an MBL ($bla_{\text{NDM-1}}$) in addition to $bla_{\text{OXA-232}}$ and the other harbored KPC-58, which has been recently described as having an insertion at position 270 of 8 amino acids (NRAPNKDD) known to increase the MIC results for this combination [21]. Meropenem-vaborbactam was active against 94.4% of the 18 isolates from 2020 producing these enzymes. Resistance to this agent was detected only in the isolate producing $bla_{\text{NDM-1}}$ plus $bla_{\text{OXA-232}}$.

DISCUSSION

Despite the many studies published evaluating the prevalence of β-lactamases, a systematic and comprehensive survey of these enzymes is challenging to conduct [7]. In a literature review intended to address the incidence of ESBL-producing *E coli* and *Klebsiella* spp clinical isolates in US hospitals, the authors evaluated >50 000 studies, but only 9 met the established criteria and were included in their final analysis [9]. This result highlights how difficult it is to reliably evaluate the prevalence of β-lactamase–producing isolates from literature reviews.

Performing prospective studies is also not a trivial task. The collection of data in multiple institutions presents its own challenges, but laboratory procedures are also diverse. Accordingly, centralized testing is considered ideal [5].

In this study, we surveyed *E coli* and *K pneumoniae* isolates consecutively collected in 56 US hospitals over 5 years. All isolates were susceptibility tested using the reference broth microdilution method. Isolates displaying resistance to key β-lactams were screened using a WGS approach. Our results highlight the continuous spread of CTX-M-15–producing isolates in US hospitals not only among *E coli*, but also among *K pneumoniae* isolates. We observed this trend in a prior study evaluating US isolates collected from 2012 to 2014 [12], but this study further documents the spread of this gene among

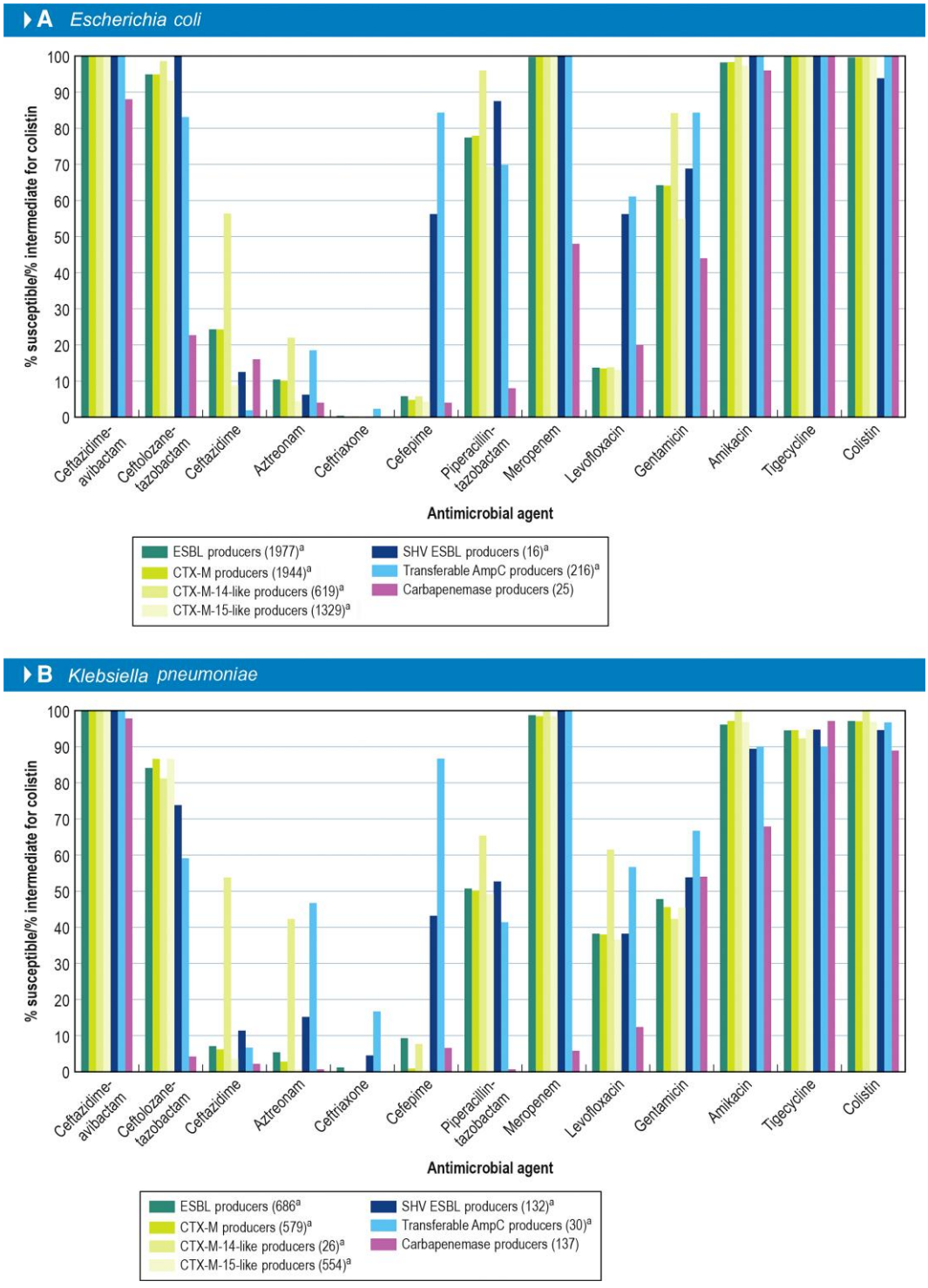

Figure 2. Antimicrobial activity of antimicrobial agents tested against β-lactamase–producing isolates during 2016–2020. [a]Carbapenemase producers not included. Abbreviation: ESBL, extended-spectrum β-lactamase.

K pneumoniae isolates. Additionally, we previously reported a decline in SHV ESBL enzymes that continued in this more recent analysis. Like our 2012–2014 survey [12], carbapenem resistance rates decreased over the course of this study, a result mostly due to a decrease of KPC-producing *K pneumoniae* in the Middle Atlantic census division. This trend was also noted by Abdallah et al [22] and Iregui et al [23], who both reported a steady decline in meropenem resistance rates among

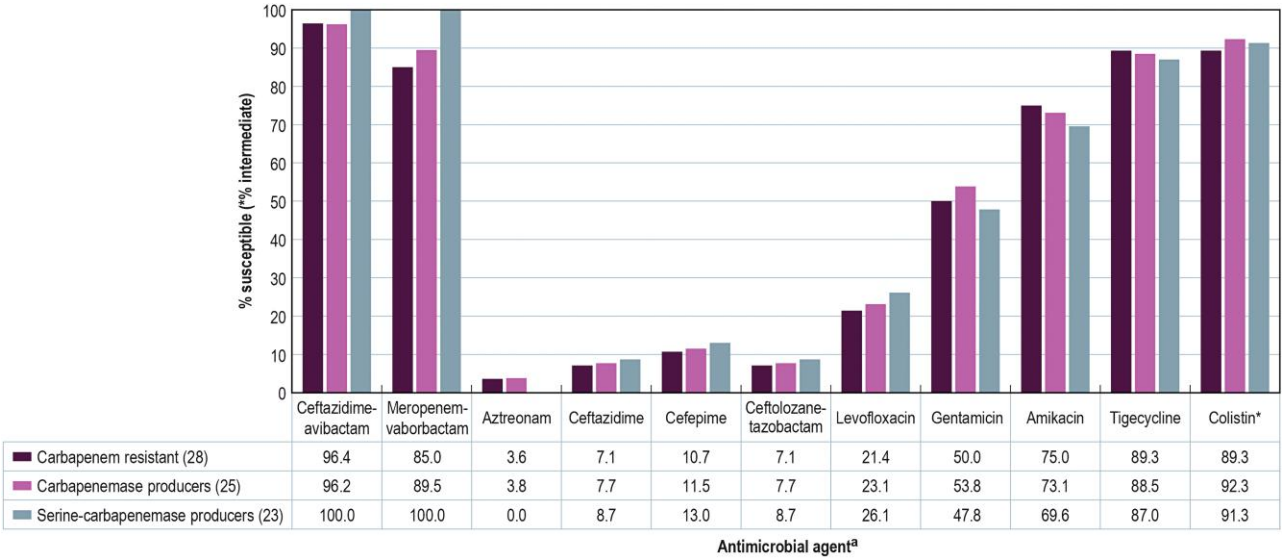

Figure 3. Antimicrobial activity of antimicrobial agents tested against carbapenem-resistant isolates from 2020. [a]Imipenem, piperacillin-tazobactam, and ceftriaxone displayed no activity against carbapenem-resistant isolates. *% intermediate.

K pneumoniae and *E coli* isolates from a New York hospital when analyzing surveillance data from 2006, 2009, 2013–2014, and 2017. Iregui et al also highlighted a decline in KPC-producing isolates and the emergence of isolates carrying $bla_{\text{NDM-5}}$ [23].

The MERINO trial challenged the use of piperacillin-tazobactam for the treatment of infections caused by ceftriaxone-resistant *E coli* and *K pneumoniae*, a marker for ESBL production [24]. The authors recommend the use of carbapenems for the treatment of these isolates; however, the increased use of carbapenems has driven higher rates of carbapenem resistance. Despite the criticisms of the MERINO trial [25], the need for alternative therapies for β-lactamase–producing isolates has been highlighted in the literature. There is a consensus that newer β-lactam/β-lactamase inhibitor combinations should be spared for the treatment of serious infections caused by CRE organisms, but it is important to continue to monitor the susceptibility profiles for these agents alongside the susceptibility profiles of established comparator agents for all β-lactamase–producing isolates.

We confirmed that the carbapenems and ceftazidime-avibactam were the most active agents tested against isolates producing ESBLs without carbapenem resistance. Meropenem-vaborbactam was also very active against the isolates from 2020. However, ceftolozane-tazobactam was slightly less active due to its lower activity against *K pneumoniae* isolates producing ESBLs. Additionally, ceftazidime-avibactam was active against 97% of the CRE isolates. The exceptions to ceftazidime-avibactam activity were MBL-producing isolates and 1 isolate producing KPC-58 that was shown to encode resistance to this combination [21]. The prevalence of isolates producing MBLs and OXA-48 enzymes is still low in US hospitals. Less than 0.1% of the isolates collected carried these enzymes.

In summary, this study reliably reports the occurrence of β-lactam–resistant phenotypes and genes in US hospitals, providing a national picture of the distribution of isolates with these characteristics. The activity of new and established antimicrobial agents is also reported and provides an understanding of resistance profiles for clinicians, policy makers, and drug developers.

Notes

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Patient consent. This study does not include factors necessitating patient consent.

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Pharmaceuticals, Mutabilis, Nabriva Therapeutics, National Institutes of Health, Novome Biotechnologies, Omnix Medical, Paratek Pharma, Pattern Bioscience, Pfizer, Prokaryotics, Pulmocide, QPEX Biopharma, Roche Holding AG, Roivant Sciences, SeLux Diagnostics, Shionogi, Sinovent Pharmaceuticals, SNIPR Biome ApS, Spero Therapeutics, Summit Therapeutics, T2 Biosystems, TenNor Therapeutics, Thermo Fisher Scientific, University of Southern California, University of Wisconsin, US Committee on Antimicrobial Susceptibility Testing, US Food and Drug Administration, Venatorx Pharmaceutics, Weill Cornell Medicine, and Wockhardt.

References

1. Bush K, Bradford PA. Beta-lactams and beta-lactamase inhibitors: an overview. Cold Spring Harb Perspect Med **2016**; 6:a025247.
2. Castanheira M, Simner PJ, Bradford PA. Extended-spectrum beta-lactamases: an update on their characteristics, epidemiology and detection. JAC Antimicrob Resist **2021**; 3:dlab092.
3. Bush K. Bench-to-bedside review: the role of beta-lactamases in antibiotic-resistant gram-negative infections. Crit Care **2010**; 14:224.
4. Sader HS, Rhomberg PR, Fuhrmeister AS, Mendes RE, Flamm RK, Jones RN. Antimicrobial resistance surveillance and new drug development. Open Forum Infect Dis **2019**; 6:S5–13.
5. Critchley IA, Karlowsky JA. Optimal use of antibiotic resistance surveillance systems. Clin Microbiol Infect **2004**; 10:502–11.
6. Pezzani MD, Tornimbene B, Pessoa-Silva C, et al. Methodological quality of studies evaluating the burden of drug-resistant infections in humans due to the WHO Global Antimicrobial Resistance Surveillance System target bacteria. Clin Microbiol Infect **2021**; 27:687–96.
7. Antimicrobial RC. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet **2022**; 399:629–55.
8. Jernigan JA, Hatfield KM, Wolford H, et al. Multidrug-resistant bacterial infections in U.S. hospitalized patients, 2012–2017. N Engl J Med **2020**; 382:1309–19.
9. McDanel J, Schweizer M, Crabb V, et al. Incidence of extended-spectrum beta-lactamase (ESBL)–producing *Escherichia coli* and *Klebsiella* infections in the United States: a systematic literature review. Infect Control Hosp Epidemiol **2017**; 38:1209–15.
10. Castanheira M, Deshpande LM, Mendes RE, Canton R, Sader HS, Jones RN. Variations in the occurrence of resistance phenotypes and carbapenemase genes among Enterobacteriaceae isolates in 20 years of the SENTRY antimicrobial surveillance program. Open Forum Infect Dis **2019**; 6:S23–33.
11. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: 32nd informational supplement, M100. Wayne, PA: CLSI; **2022**.
12. Castanheira M, Mendes RE, Jones RN, Sader HS. Changes in the frequencies of beta-lactamase genes among Enterobacteriaceae isolates in U.S. hospitals, 2012 to 2014: activity of ceftazidime-avibactam tested against beta-lactamase-producing isolates. Antimicrob Agents Chemother **2016**; 60:4770–7.
13. Sader HS, Flamm RK, Carvalhaes CG, Castanheira M. Antimicrobial susceptibility of *Pseudomonas aeruginosa* to ceftazidime-avibactam, ceftolozane-tazobactam, piperacillin-tazobactam, and meropenem stratified by U.S. census divisions: results from the 2017 INFORM program. Antimicrob Agents Chemother **2018**; 62:e01587.
14. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: 11th ed. M07. Wayne, PA: CLSI; **2018**.
15. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: 31st informational supplement, M100. Wayne, PA: CLSI; **2021**.
16. US Food and Drug Administration. Antibacterial susceptibility test interpretive criteria. **2020**. https://www.fda.gov/drugs/development-resources/antibacterial-susceptibility-test-interpretive-criteria. Accessed 15 March 2021.
17. Bankevich A, Nurk S, Antipov D, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol **2012**; 19:455–77.
18. Camacho C, Coulouris G, Avagyan V, et al. BLAST+: architecture and applications. BMC Bioinformatics **2009**; 10:421.
19. Castanheira M, Doyle TB, Kantro V, Mendes RE, Shortridge D. Meropenem-vaborbactam activity against carbapenem-resistant Enterobacterales isolates collected in U.S. hospitals during 2016 to 2018. Antimicrob Agents Chemother **2020**; 64:e01951.
20. Hanahan D. Studies on transformation of *Escherichia coli* with plasmids. J Mol Biol **1983**; 166:557–80.
21. Castanheira MD, Lalitagauri MM, Rodrigo ED, Timothy BS, Helio S. Prevalence of carbapenemase genes among carbapenem-nonsusceptible Enterobacterales collected in US hospitals in a 5-year period and activity of ceftazidime-avibactam and comparator agents. JAC Antimicrob Resist **2022**; 4:dlac098.
22. Abdallah M, Olawole O, Cortes C, et al. Rise and fall of KPC-producing *Klebsiella pneumonia* in New York City. J Antimicrob Chemother **2016**; 71:2945–8.
23. Iregui A, Ha K, Meleney K, Landman D, Quale J. Carbapenemases in New York City: the continued decline of KPC-producing *Klebsiella pneumoniae*, but a new threat emerges. J Antimicrob Chemother **2018**; 73:2997–3000.
24. Harris PNA, Tambyah PA, Lye DC, et al. Effect of piperacillin-tazobactam vs meropenem on 30-day mortality for patients with *E coli* or *Klebsiella pneumoniae* bloodstream infection and ceftriaxone resistance: a randomized clinical trial. JAMA **2018**; 320:984–94.
25. Rodriguez-Bano J, Gutierrez-Gutierrez B, Pascual A. CON: carbapenems are NOT necessary for all infections caused by ceftriaxone-resistant Enterobacterales. JAC Antimicrob Resist **2021**; 3:dlaa112.