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Prevalence of β-Lactamase-Encoding Genes among *Enterobacteriaceae* Bacteremia Isolates Collected in 26 U.S. Hospitals: Report from the SENTRY Antimicrobial Surveillance Program (2010)

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Enterobacteriaceae **bacteremia isolates (*n* = 195; 6.4% overall) collected from 26 U.S. hospitals located in 20 states were screened for various β-lactamase classes. A total of 175 isolates carried one to eight acquired β-lactamase genes of 44 types that were detected in 55 combinations. Eighty-five (43.6%) strains carried bla_{CTX-M}, and $bla_{CTX-M-15}$ was the most prevalent (33.8%). Genes encoding OXA-1/30 (often associated with $bla_{CTX-M-15}$), CMY-2, SHV extended-spectrum β-lactamase (ESBLs), and TEM-1 were also prevalent. Among 33 carbapenem-resistant strains, 28 carried bla_{KPC-2} or bla_{KPC-3} (17 and 11 strains, respectively), and those were recovered mostly in the New York City area (16 strains) and Houston, TX (9 strains). Fourteen new SHV variants were identified among *Klebsiella pneumoniae* isolates carrying one or multiple SHV alleles, three carrying G238S and/or E240K amino acid alterations that confer ESBL activity. Only two of eight *K. oxytoca* isolates carried acquired β-lactamases, but most had mutations on the bla_{OXY} promoter region, and three new OXY-encoding genes were characterized. Concordance between a commercial nucleic acid-based microarray (Check-MDR CT101) and reference methods was noted for 105/109 (97.2%) strains. Thirty-two strains having genes that are not targeted by the commercial system were detected (OXA ESBLs, PER, PSE, or intrinsic genes). Overall, a great variety of enzymes were observed, with numerous strains carrying multiple genes. Rates of CTX-M-producing strains appear to be increasing in U.S. hospitals (26.6% in 2007 to 43.8% for 2010) participating in the SENTRY Program. Furthermore, the Check-Points system seems to be a reliable, robust, and user-friendly assay for detection of enzyme-mediated resistance.**

β-Lactamases have been recognized as the main cause of cephalosporin resistance among members of the *Enterobacteriaceae* family. Several types of acquired β-lactamases conferring resistance to different β-lactam agents have been described among these organisms, and extended-spectrum β-lactamases (ESBLs) that confer elevated MICs to penicillins, cephalosporins, and aztreonam (1) are becoming very prevalent in the nosocomial and community settings. CTX-M, TEM, and SHV variants are the most numerous and prevalent (1–3); however, a variety of other enzymes such as VEB, GES/IBC, PER, BEL, and oxacillinases with ESBL activity have been described worldwide (1). Furthermore, strains carrying ESBL-encoding genes are often resistant to other antimicrobial classes (4, 5), such as trimethoprim-sulfamethoxazole, aminoglycosides, and fluoroquinolones.

Acquired Ambler class C cephalosporinases, also called plasmid-mediated AmpCs, have emerged through the mobilization of chromosomal genes of inducible AmpC β-lactamases onto plasmids (6). When transferred into other organisms such as *Escherichia coli* and *Klebsiella pneumoniae*, these cephalosporinases have substrate profiles similar to those of the parent chromosomal enzymes but in most cases differ in having constitutively expressed enzyme activity. Additionally, metallo-β-lactamases, such as NDM, KPC, and OXA-48 carbapenemases, have been increasingly described among *Enterobacteriaceae* strains, and these enzymes can cause resistance to the vast majority of β-lactams (7, 8).

In this study, we evaluated the presence of ESBLs, acquired cephalosporinases, broad-spectrum β-lactamases, and carbapenemases among 195 cephalosporin-resistant *Enterobacteriaceae* strains collected from clinical invasive infections (bacteremias) in 26 U.S. hospitals during 2010 as part of the SENTRY Antimicrobial Surveillance Program. In this process, we identified three bla_{OXY} and 14 bla_{SHV} new variants. Additionally, we evaluated an *in vitro* nucleic acid-based microarray (Check-MDR CT101 kit; Check-Points, Wageningen, Netherlands) with a large subset of the described strains.

MATERIALS AND METHODS

Bacterial isolates and antimicrobial susceptibility testing. A total of 3,061 *Enterobacteriaceae* bloodstream isolates were consecutively collected from 26 hospitals located in 20 U.S. states during 2010 as part of the SENTRY Program. Only one isolate per patient from documented bloodstream infections was included in the study. Species identification was confirmed by standard biochemical tests and the Vitek System (bioMérieux, Hazelwood, MO), when necessary. All isolates were susceptibility tested using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI) (9). Categorical interpretations for all antimicrobials were those found in CLSI document M100-S22 (10), and quality control (QC) was performed using *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within specified ranges as published by the CLSI (10).

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Genotypic detection of β-lactamases. *E. coli*, *Klebsiella*, and *Proteus mirabilis* strains were selected based on the CLSI criteria for ESBL epidemiological screening (10). Species that may hyperexpress constitutive AmpC enzymes (*Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., and indole-positive *Proteae*) displaying cefepime MICs of ≥2 μg/ml were also selected for this study. PCR screening was performed for bla_{TEM} and bla_{SHV} in singleplex reactions using a high-fidelity master mix or in a combination of multiplex reactions for bla_{CTX-M}, bla_{GES}, bla_{VEB}, bla_{PER}, bla_{PSE}, and genes for oxacillinases with an ESBL spectrum (bla_{OXA-2}, bla_{OXA-10}, and bla_{OXA-30} groups; bla_{OXA-18}; and bla_{OXA-45}). Additionally, bla_{CMY}, $bla_{FOX-1-7}$, $bla_{ACC-1-4}$, $bla_{ACT-1-7}$, $bla_{DHA-1-3}$, bla_{LAT-1}, $bla_{MIR-1-5}$, and $bla_{MOX-1-7}$ were also amplified in a multiplex reaction modified to include newer variants from the work of Perez-Perez and Hanson (11). Oligonucleotides used in this study are listed in the supplemental material.

Additionally, isolates with reduced susceptibility to imipenem or meropenem (MIC, ≥2 μg/ml) were screened by PCR for presence of the following carbapenemase genes: bla_{IMP}, bla_{VIM}, bla_{SPM-1},bla_{KPC}, bla_{NDM}, bla_{SME}, bla_{IMI}, bla_{NMC-A}, bla_{GES}, and bla_{OXA-48}.

Amplicons were sequenced on both strands, and the nucleotide sequences and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, WI). Sequences were compared to others available via Internet sources (http://www.ncbi.nlm.nih.gov/blast/).

Chromosomal AmpC of *E. coli* and *Enterobacter cloacae* strains was evaluated using RNA preparations and amplification as previously described (12), using a quantitative real-time PCR approach and custom-designed oligonucleotides showing efficiencies of >98.0%.

Characterization of new OXY variants and *Klebsiella oxytoca* strain types. *K. oxytoca* strains had the bla_{OXY} and associated promoter regions sequenced (13). Amplicons containing the β-lactamase open reading frame and promoter region were cloned into pPCRScriptCam SK+ (Stratagene, Santa Clara, CA). The colonies obtained after transformation in XL10-Gold Kan ultracompetent *E. coli* were selected on plates containing 30 μg/ml of chloramphenicol. The presence and orientation of inserts were confirmed by PCR and sequencing, and MIC testing was performed as described above.

K. oxytoca clinical isolates were also analyzed for phylogenetic relationships using the method described by Fevre et al. (14), which included the sequencing and analysis of *rpoB*, *gyrA*, *gapDH*, and *rrs*.

Identification of new bla_{SHV} variants. Isolates displaying double peaks in bla_{SHV} initial sequencing and one isolate carrying a single novel bla_{SHV} gene had amplicons produced with high-fidelity *Taq* DNA polymerase master mix cloned into TOPO (Life Technologies, Carlsbad, CA). Ten to 20 colonies confirmed to carry the insert were sequenced, and new SHV types were identified. Confirmation of the presence of multiple bla_{SHV} copies was performed by partial digestion with S1 nuclease and ICeuI digestion of total cellular DNA embedded in 1% agarose plugs. Electrophoresis was performed on a CHEF-DR II (Bio-Rad, Richmond, CA), and DNA gels were transferred to nylon membranes by Southern blotting and hybridized with a digoxigenin-labeled (Roche Diagnostics GmbH, Mannheim, Germany) bla_{SHV}-generic probe.

Commercial microarray. A subset (109 strains) of *E. coli* (62 strains), *K. pneumoniae* (45 strains), and *K. oxytoca* (3 strains) was additionally tested using the Check-MDR CT101 kit (Check-Points, Wageningen, Netherlands). The assay was performed according to the manufacturer's instructions. This kit has the capabilities to detect CTX-M groups 1, 2, 8 plus 25, and 9, TEM wild type (WT) and ESBL, SHV WT and ESBL, ACC, ACT/MIR, CMYII, DHA, FOX, KPC, and NDM-1. DNA from isolates showing discordant results compared to PCR and sequencing were sent to the manufacturer and retested.

IEF. Protein preparations were obtained with BugBuster (Novagen, Darmstadt, Germany) and submitted to isoelectric focusing (IEF) using precast Novex gels and an electrophoresis system (Life Technologies) according to the manufacturer's instructions for those isolates showing discrepant results between PCR and sequencing and the Check-Points microarray. Gels were stained using nitrocefin, and the isoelectric point (pI) was determined by linear regression using a combination of known β-lactamases and a prestained protein marker (Bio-Rad).

Nucleotide sequence accession numbers. Nucleotide sequences encoding new β-lactamase enzymes identified in this study were submitted to GenBank and have the following accession numbers: OXY-2-11, JX559324; OXY-2-12, JX559325; OXY-2-13, JX559327; SHV-147, JX121114; SHV-148, JX121115; SHV-149, JX121116; SHV-150, JX121117; SHV-151, JX121118; SHV-153, JX121120; SHV-154, JX121121; SHV-155, JX121122; SHV-156, JX121123; SHV-158, JX121125; SHV-159, JX121126; SHV-161, JX121128; SHV-163, JX121130; and SHV-165, JX121130.

RESULTS AND DISCUSSION

ESBL prevalence and β-lactamases detected. Among *Enterobacteriaceae* blood culture isolates received from U.S. hospitals, 195 (6.4% of the total) strains had elevated cephalosporins and/or aztreonam MIC results and were selected for further evaluation. These strains belonged to the following bacterial species: *E. coli* (81 isolates), *K. pneumoniae* (71 isolates), *Enterobacter cloacae* (25 isolates), *K. oxytoca* (8 isolates), *P. mirabilis* (5 isolates), and *Citrobacter braakii*, *Enterobacter aerogenes*, *Enterobacter gergoviae*, *Proteus vulgaris*, and *Serratia marcescens* (1 isolate of each). These isolates were detected in all 26 hospitals surveyed. Isolates were resistant to cephalosporins, including cefepime (48.7% of isolates nonsusceptible). The activity of carbapenems varied from 77.4% susceptibility for ertapenem to 84.6% susceptibility for doripenem. Amikacin and tigecycline were the only two compounds displaying coverage at >90% of the isolates tested (data not shown).

A total of 175 strains carried acquired β-lactamase-encoding genes, and among those, 157 isolates harbored genes encoding ESBLs, carbapenemases, and/or acquired AmpC enzymes. Forty-four types of β-lactamases and 55 different enzyme combinations (two to nine β-lactamases) were observed among these isolates (Table 1). Among the 26 (13.3%) isolates that displayed no acquired β-lactamase-encoding genes, these included 11 *Enterobacter* isolates, 7 *E. coli* isolates, six *K. oxytoca* strains, and 1 isolate each of *C. braakii* and *P. stuartii*. Among these isolates, six *E. coli* isolates displayed borderline MICs for ceftriaxone, aztreonam, or ceftazidime, and screening with more than one agent, as suggested in the CLSI document (10), would have minimized the selection of these strains. Follow-up testing to evaluate the activity of intrinsic β-lactamases was performed on *E. cloacae*, *E. coli*, and *K. oxytoca* strains. The expression of the chromosomal AmpC was determined for nine *E. cloacae* strains (two failed to amplify using the assay designed) and one *E. coli* strain. All *E. cloacae* strains had AmpC expression 1,000 times greater than that of the comparator control strain, *E. cloacae* ATCC 700323 (data not shown), thus hyperexpressing the chromosomal cephalosporinase. The *E. coli* strain had AmpC expression similar to that of the control strain (ratio of expression compared to *E. coli* ATCC 25922 was 2.67).

The most prevalent β-lactamase gene detected by PCR and sequencing was $bla_{CTX-M-15}$, which was observed among 66 (33.8%) strains from three bacterial species (41 *E. coli*, 24 *K. pneumoniae*, and one *S. marcescens* strain) collected from 21 hospitals located in 16 states. This gene was detected alone (9 strains) or in 12 combinations with one to four additional β-lactamase genes (Table 1). CTX-M-15-producing isolates often carry $bla_{OXA-1/30}$ (32 strains), but other extended- and broad-spectrum enzymes were commonly observed among these strains. Four CTX-M-15-

TABLE 1 Enzyme combinations detected among *Enterobacteriaceae* strains collected from U.S. hospitals during 2010

β-Lactamases	No. of isolates	State(s) (no. of isolates)	Bacterial species (no. of isolates)
Combinations including carbapenemases			
KPC-2, CTX-M-15, SHV-1, TEM-1	3	Texas (3)	*K. pneumoniae* (3)
KPC-2, OXA-2, OXY-2-4	1	Virginia (1)	*K. oxytoca* (1)
KPC-2, SHV-1, TEM-1	2	New York (1), Texas (1)	*K. pneumoniae* (2)
KPC-2, SHV-11	1	New York (1)	*K. pneumoniae* (1)
KPC-2, SHV-11, TEM-1	5	Texas (5)	*K. pneumoniae* (5)
KPC-2, TEM-1	4	Massachusetts (2), New York (1), Virginia (1)	*E. cloacae* (3), *K. pneumoniae* (1)
KPC-3, CTX-M-15, OXA-1/30, SHV-11, TEM-1	1	New York (1)	*K. pneumoniae* (1)
KPC-3, SHV-11, TEM-1	9	Michigan (3), New Jersey (2), New York (4)	*K. pneumoniae* (9)
KPC-3, SHV-12, TEM-1	1	Virginia (1)	*E. cloacae* (1)
KPC-3, TEM-1	1	New Jersey (1)	*E. cloacae* (1)
Combinations including ESBLs			
CTX-M-14, SHV-11, TEM-1	2	Wisconsin (2)	*K. pneumoniae* (2)
CTX-M-14, SHV-63, TEM-1	1	Florida (1)	*K. pneumoniae* (1)
CTX-M-14, TEM-1	4	Colorado (1), Michigan (1), New York (2)	*E. coli* (4)
CTX-M-15, OXA-1/30	17	Colorado (1), Florida (2), Hawaii (1), Kentucky (1), Michigan (3), New Jersey (1), New York (5), Texas (1), Virginia (2)	*E. coli* (17)
CTX-M-15, OXA-1/30, PSE-like[a]	1	Nebraska (1)	*E. coli* (1)
CTX-M-15, OXA-1/30, SHV-1	1	New York (1)	*K. pneumoniae* (1)
CTX-M-15, OXA-1/30, SHV-1, TEM-1	2	Indiana (1), New York (1)	*K. pneumoniae* (2)
CTX-M-15, OXA-1/30, SHV-11, TEM-1	4	Kentucky (1), New York (2), Ohio (1)	*K. pneumoniae* (4)
CTX-M-15, OXA-1/30, TEM-1	6	Iowa (1), Michigan (3), New York (1), Ohio (1)	*E. coli* (6)
CTX-M-15, SHV-1	3	Kentucky (1), New Jersey (1), Texas (1)	*K. pneumoniae* (3)
CTX-M-15, SHV-1, TEM-1	9	Michigan (1), New Jersey (1), Texas (7)	*K. pneumoniae* (8), *S. marcescens* (1)
CTX-M-15, SHV-11	2	Michigan (2)	*K. pneumoniae* (2)
CTX-M-15, TEM-1	8	Florida (1), Kentucky (1), Massachusetts (1), Nebraska (1), New York (2), Utah (1), Vermont (1)	*E. coli* (8)
CTX-M-27, OXA-1/30	1	New York (1)	*E. coli* (1)
CTX-M-27, SHV-11, TEM-1	2	Massachusetts (1), New York (1)	*E. coli* (1), *K. pneumoniae* (1)
CTX-M-27, TEM-1	1	Vermont (1)	*E. coli* (1)
CTX-M-91, TEM-1	1	New Jersey (1)	*P. mirabilis* (1)
PER-1, SHV-12, TEM-1	1	New York (1)	*P. vulgaris* (1)
SHV-2, TEM-1	1	Kentucky (1)	*K. pneumoniae* (1)
SHV-2, SHV-1, SHV-141	1	Massachusetts (1)	*K. pneumoniae* (1)
SHV-2, SHV-1, SHV-153	1	Kentucky (1)	*K. pneumoniae* (1)
SHV-5, SHV-1, SHV-149, SHV-150, SHV-151, SHV-154, SHV-161, OXA-1/30, TEM-1	1	New York (1)	*K. pneumoniae* (1)
SHV-5, SHV-1, SHV-11, SHV-147, SHV-148, OXA-2, TEM-1	1	New York (1)	*K. pneumoniae* (1)
SHV-5, SHV-75	1	New York (1)	*K. pneumoniae* (1)
SHV-5, SHV-11, TEM-1	1	Ohio (1)	*K. pneumoniae* (1)
SHV-7, OXA-2, TEM-1	2	Kentucky (2)	*E. cloacae* (2)
SHV-7,OXA-9, TEM-1	1	Virginia (1)	*E. coli* (1)
SHV-7, TEM-1	1	Virginia (1)	*E. coli* (1)
SHV-12, OXY-2-12, TEM-1	1	Ohio (1)	*K. oxytoca* (1)
SHV-12, SHV-11	1	New Jersey (1)	*K. pneumoniae* (1)
SHV-12, SHV-11, SHV-141, SHV-161, SHV-163, TEM-1	1	Massachusetts (1)	*K. pneumoniae* (1)
SHV-12, SHV-11, SHV-155, SHV-156, SHV-160	1	Ohio (1)	*K. pneumoniae* (1)
SHV-12, SHV-11, SHV-158, SHV-159	1	New York (1)	*K. pneumoniae* (1)
SHV-12, SHV-11, TEM-1	1	Massachusetts (1)	*K. pneumoniae* (1)
SHV-12, TEM-1	5	Arkansas (1)	*E. coli* (1), *E. coli* (1), *K. pneumoniae* (3)
TEM-155, SHV-11	1	New York (1)	*K. pneumoniae* (1)
Combinations including acquired cephalosporinases			
CMY-2, CTX-M-14	1	New York (1)	*E. coli* (1)
CMY-2, SHV-33	1	Vermont (1)	*K. pneumoniae* (1)
CMY-2, TEM-1	5	Colorado (1), Kentucky (1), Michigan (1), New Jersey (1), Texas (1)	*E. coli* (5)
CMY-like,[b] TEM-1	1	Iowa (1)	*E. coli* (1)
DHA-1, TEM-1	1	Hawaii (1)	*P. mirabilis* (1)
FOX-5, PSE-like[a]	3	Florida (1), Massachusetts (1), Virginia (1)	*E. cloacae* (1), *K. pneumoniae* (1), *P. mirabilis* (1)
FOX-5, SHV-11	1	Wisconsin (1)	*K. pneumoniae* (1)
FOX-5, PSE-like,[a] SHV-11	1	Kentucky (1)	*K. pneumoniae* (1)

[a] bla_{PSE} sequencing coverage did not allow differentiation of PSE-1 and PSE-4.
[b] New CMY variant similar to CMY-55.

producing *K. pneumoniae* strains harbor KPC-encoding genes: one KPC-3 producer from the New York area and three KPC-2 producers from Houston, TX.

Nineteen isolates carried other CTX-M variants, including CTX-M-14 (12 isolates; 9 *E. coli* and 3 *K. pneumoniae*), CTX-M-27 (6 isolates; 5 *E. coli* and 1 *K. pneumoniae*), and CTX-M-91 (1 *P. mirabilis*). Overall, CTX-M-producing isolates were detected in 23 hospitals, and among 11 hospitals, multiple CTX-M types were noted.

Due to its association to $bla_{CTX\text{-}M\text{-}15}$, the gene encoding OXA-1/30 was the second most prevalent gene detected. This gene was observed in a total of 34 isolates (25 *E. coli* and 9 *K. pneumoniae*), only 2 not carrying $bla_{CTX\text{-}M\text{-}15}$ and 1 harboring $bla_{CTX\text{-}M\text{-}27}$. SHV ESBLs were noted in 32 strains, and the most common type was

SHV-12 (13 strains), but SHV-5, SHV-7, SHV-2, and SHV-30 (5, 4, 3, and 3 isolates, respectively) were also detected. One *K. pneumoniae* strain displayed a single new SHV variant, and this strain and 10 *K. pneumoniae* strains displaying double peaks during sequence analysis of bla_{SHV} amplicons were further characterized (see below).

Among other ESBL enzymes, PER-1 was noted in three isolates (two *E. coli* and one *P. vulgaris*), and TEM-12 and TEM-155 were found in one strain each (*E. coli* and *P. mirabilis*, respectively). Additionally, acquired AmpC enzymes were observed in 19 strains: 12 CMY-2 (11 *E. coli* and 1 *K. pneumoniae*), 5 FOX-5 (3 *K. pneumoniae*, 1 *P. mirabilis*, and 1 *E. cloacae*), 1 DHA-1 (*P. mirabilis*), and 1 new CMY variant (*E. coli*). The gene for the last was most similar to that for CMY-55.

Broad-spectrum β-lactamases TEM-1, SHV-11, and SHV-1 were the most prevalent among this class and were detected in 99, 38, and 26 strains, respectively, alone or in combination with other enzymes. Other β-lactamase-encoding genes, such as bla_{PSE}-like (encoding PSE-1 or PSE-4 [not able to differentiate due to sequencing coverage] in five strains from four bacterial species), bla_{OXA-2} (four strains from three bacterial species), and bla_{OXA-9} (one *E. coli* strain) were detected, and those were routinely associated with other genes encoding β-lactamases.

Thirty-three carbapenem-nonsusceptible isolates (imipenem and/or meropenem MIC, ≥2 μg/ml) were included in the study, and 28 of these strains carried KPC-encoding genes. bla_{KPC-2} was found among 17 strains, whereas bla_{KPC-3} was noted in 11 strains. These isolates were recovered in nine hospitals located in six states, four in the New York City area (16 strains); Houston, TX (9 strains); and Detroit, MI (3 strains). KPC-2 was detected alone in one *E. cloacae* strain, but all the remaining KPC-2 and KPC-3 producers carried multiple enzymes, in a total of 10 different combinations (Table 1). Isolates with the same β-lactamase content were observed within hospitals in New York and Texas, but whether these isolates are clonal or the same genetic element is being mobilized to different strains remains to be elucidated. No other carbapenemase-encoding genes were observed in this collection.

Characterization of OXY enzymes. Among eight *K. oxytoca* isolates, representing 7.4% overall of submitted isolates from this species, sequencing of the OXY-encoding gene revealed six bla_{OXY} types (Table 2). Two isolates carried the gene encoding OXY-1-2 and belonged to the KoI group according to Fevre et al. (14). All other isolates belonged to the KoII phylogenetic group and carried OXY-2 types: one strain carried the gene encoding OXY-2-4, and three new OXY-2 variants, named OXY-2-11 (two strains), OXY-2-12 (one strain), and OXY-2-13 (two strains; nomenclature according Institute Pasteur curator), were identified.

Sequencing of the bla_{OXY} upstream region demonstrated that four of the eight strains had mutations in the −10 promoter region leading to the increased expression of bla_{OXY} (13) (Table 2). These isolates carried genes encoding OXY-1-2, OXY-2-4, OXY-2-11, or OXY-2-13 (one each). None of the isolates had changes in the −35 promoter sequence, and all strains had an identical 17-bp spacer. Isolates showing no mutations in the promoter displayed modestly elevated MICs for most β-lactams or produced other β-lactamases (Table 2). Two *K. oxytoca* isolates carried additional β-lactamase genes: one strain from Akron, OH, carried genes encoding SHV-12 and TEM-1 in addition to the OXY-2-12 gene that displayed no alterations in the promoter region. Another isolate from Charlottesville, VA, displayed elevated carbapenem MICs (imipenem and meropenem MICs, 4 μg/ml) and harbored bla_{KPC-2} and bla_{OXA-2}. This strain carried the gene encoding OXY-2-4 and had a mutation in the −10 promoter region (GATA[G→A]T) that has been shown to increase the expression of downstream genes >4-fold (13).

Susceptibility results for bla_{OXY} expressed in an *E. coli* background showed notable differences in the MICs for the compounds tested (Table 2). Aztreonam MICs were elevated among all recombinant strains (MIC, 8 to >16 μg/ml), but a difference could be observed in the two isolates carrying the gene encoding OXY-2-11 (H155R and D199N mutations) that had distinct promoter sequences. The isolate showing a stronger −10 promoter region (GATAAT) had at least 2-fold-greater aztreonam MICs in repeated testing. This strain also displayed greater ceftriaxone and cefepime MICs than did its peer harboring the standard −10 promoter sequence (GATAAT). OXY-2-11-producing *E. coli* displayed cefoxitin MICs greater than with all other variants even though this gene was not regulated by a stronger promoter sequence. Ceftazidime MICs were slightly greater in the two isolates producing OXY-1-2 and OXY-2-4 under a stronger promoter influence; this β-lactam is not well hydrolyzed by OXY enzymes (14).

Identification of new SHV variants. All 10 *K. pneumoniae* isolates displaying double peaks on bla_{SHV} sequences in multiple independent reactions had bla_{SHV} amplicons successfully cloned. According to the sequencing of multiple recombinant colonies, these isolates carried two to seven bla_{SHV} variants (Table 3) and among those, 13 new genes were identified. New SHV variants had one to three amino acid alterations compared to SHV-1, and three variants had mutations in positions 238 and 240 that are known to expand the hydrolytic profile to the cephalosporins (15): SHV-153, SHV-154, and SHV-163. In all cases, at least one ESBL SHV-encoding gene was detected in those isolates (Table 2). S1 nuclease and ICeuI experiments confirmed that those isolates had multiple copies of the SHV gene in plasmids and/or chromosomes (see the supplemental material). In addition, a new SHV enzyme detected on the plasmid of one *K. pneumoniae* isolate was named SHV-157 and had a V75M amino acid substitution compared to SHV-11, the closest variant.

Comparison of reference PCR and sequencing methods to Check-Points microarray. A total of 109 *E. coli* (62 strains) and *Klebsiella* spp. (44 *K. pneumoniae* and 3 *K. oxytoca* strains) isolates were also evaluated using the Check-MDR CT101 kit, and this commercial microarray displayed an excellent correlation with reference PCR followed by amplicon sequencing for the genes tested by both methods, with only four discrepant results (Table 4). Two isolates displayed TEM WT results on Check-MDR CT101 but not by PCR sequencing, whereas two isolates displayed negative TEM WT results and bla_{TEM-1} was repeatedly detected by the reference methods. These isolates were shared with the kit manufacturer and were retested in their facility, displaying identical outcomes, and were confirmed by IEF as part as this study, validating the reference PCR and sequencing results. Sensitivity and specificity of Check-MDR CT101 compared to PCR and sequencing for the tests included in both methodologies were acceptable: 0.98 and 0.99, respectively.

Among 34 isolates (Table 4), genes that are not included in the Check-MDR CT101 kit were detected by reference amplification and sequencing, but in all instances the overall result for ESBL

TABLE 2 Demographic information, selected susceptibility testing results, and β-lactamases present among ESBL phenotype-positive *K. oxytoca* strains collected from blood cultures in U.S. hospitals during 2010

OXY variant[a]	Phylogenetic group	City and state	OXY variant amino acid substitution[b]	−10 promoter sequence	MIC (μg/ml)									Additional β-lactamases
					Ampicillin	Aztreonam	Cefoxitin	Ceftriaxone	Ceftazidime	Cefepime	Piperacillin-tazobactam	Imipenem	Meropenem	
K. oxytoca clinical strains														
OXY-1-2	KoI	Ewa Beach, HI	C861G silent mutation	GATAGT	>8	>16	≤2	4	0.12	≤0.12	64	≤0.12	≤0.12	
OXY-1-2	**KoI**	**Charlottesville, VA**		**GATAAT**	**>8**	**8**	**>16**	**>8**	**2**	**2**	**8**	**1**	**≤0.12**	
OXY-2-4	**KoII**	**Charlottesville, VA**	**A15 deletion, H155R**	**GATAAT**	**>8**	**>16**	**>16**	**>8**	**8**	**8**	**>64**	**4**	**4**	**KPC-2, OXA-2**
OXY-2-11	KoII	New Brunswick, NJ	H155R, D199N	GATAGT	>8	2	16	0.25	0.5	0.25	8	≤0.12	≤0.12	
OXY-2-11	**KoII**	**New York, NY**	**H155R, D199N**	**GATAAT**	**>8**	**>16**	**>16**	**>8**	**1**	**2**	**>64**	**0.25**	**≤0.12**	
OXY-2-12	KoII	Akron, OH	H155R, D255N	GATAGT	>8	8	≤2	4	4	0.25	1	≤0.12	≤0.12	SHV-12, TEM-1
OXY-2-13	**KoII**	**Burlington, MA**	**G143H**	**TATAGT**	**>8**	**>16**	**≤2**	**>8**	**0.25**	**4**	**>64**	**≤0.12**	**≤0.12**	
OXY-2-13	KoII	Milwaukee, WI	G143H	GATAGT	>8	1	16	0.5	0.5	≤0.12	16	≤0.12	≤0.12	
E. coli carrying bla_{OXY} plasmid constructs														
OXY-1-2	**NA[c]**	**NA**		**GATAAT**	**>8**	**>16**	**2**	**>8**	**1**	**2**	**>64**	**0.25**	**≤0.06**	
OXY-2-4	**NA**	**NA**	**A15 deletion, H155R**	**GATAAT**	**>8**	**>16**	**4**	**>8**	**1**	**8**	**>64**	**0.5**	**≤0.06**	
OXY-2-11	NA	NA	H155R, D199N	GATAGT	>8	8	8	1	0.25	≤0.5	32	0.25	≤0.06	
OXY-2-11	**NA**	**NA**	**H155R, D199N**	**GATAAT**	**>8**	**>16**	**4**	**>8**	**0.5**	**4**	**>64**	**0.25**	**≤0.06**	
OXY-2-12	NA	NA	H155R, D255N	GATAGT	>8	16	16	8	0.5	2	>64	0.25	≤0.06	
OXY-2-13	**NA**	**NA**	**G143H**	**TATAGT**	**>8**	**>16**	**16**	**>8**	**0.5**	**4**	**>64**	**0.25**	**≤0.06**	
OXY-2-13	NA	NA	G143H	GATAGT	>8	>16	2	8	0.25	2	>64	0.25	≤0.06	
Vector alone	NA	NA	NA	NA	2	≤0.12	4	≤0.25	≤0.12	≤0.12	1	0.25	≤0.12	

[a] Isolates with stronger promoters are in bold. New variants are underlined.
[b] Compared to the closest variant (OXY-1-1 or OXY-2-1).
[c] NA, not applicable.

TABLE 3 *K. pneumoniae* strains with double peaks in the SHV sequences and/or carrying new SHV variants that were submitted to cloning and sequencing of multiple colonies

β-Lactamase content of clinical isolate[a]	New SHV types and amino acid alterations compared to SHV-1[b]	Check-MDR CT101 results	City and state	MIC (μg/ml)						
				Aztreonam	Ceftazidime	Ceftriaxone	Cefepime	Piperacillin/ Tazobactam	Imipenem	Meropenem
OXA-1/30, SHV-1, **SHV-5**, SHV-149, SHV-150, SHV-151, SHV-154, SHV-161, TEM-1	SHV-149 Q39R, R43S, F151S SHV-150 A248V **SHV-154 R43S, G238S, E240K** SHV-161 R43S	SHV 238S + 240K SHV WT (238G + 240E) TEM WT	New York, NY	>16	>32	>8	1	>64	0.25	≤0.12
OXA-2-like, SHV-1, **SHV-5** SHV-11, SHV-147, SHV-148, TEM-1	SHV-147 T71A, P269S SHV-148 R292Q	SHV 238S + 240K SHV WT (238G + 240E) TEM WT	New York, NY	>16	>32	>8	2	>64	≤0.12	≤0.12
SHV-11, **SHV-12**		SHV 238S + 240K SHV WT (238G + 240E)	New Brunswick, NJ	>16	>32	>8	4	16	≤0.12	≤0.12
SHV-11, **SHV-12**, SHV-158, SHV-159	SHV-158 L35Q, T53A SHV-159 L35Q, D213G	SHV 238S + 240K SHV WT (238G + 240E)	New York, NY	>16	>32	>8	>16	>64	≤0.12	≤0.12
SHV-1, **SHV-2**, SHV-141		SHV 238S SHV WT (238G)	West Roxbury, MA	4	4	8	1	16	≤0.12	≤0.12
SHV-11, **SHV-12**, SHV-155, SHV-156, SHV-160	SHV-155 L35Q, R292Q SHV-156 L35Q, L91P, A150T SHV-160 L35Q, S38G	SHV 238S + 240K SHV WT (238G + 240E)	Akron, OH	>16	>32	>8	4	>64	≤0.12	≤0.12
SHV-5, SHV-75		SHV 238S + 240K SHV WT (238G + 240E) TEM WT	Rochester, NY	8	16	4	0.25	2	0.25	≤0.12
SHV-1, **SHV-2**, SHV-153	**SHV-153 G238S, A273V**	SHV 238S, SHV WT (238G)	Lexington, KY	8	16	>8	4	32	≤0.12	0.25
SHV-157[c]	SHV-157 L35Q, V75 M	SHV WT	Charlottesville, VA	4	8	8	2	16	≤0.12	≤0.12
SHV-11, **SHV-12**, TEM-1		238S + 240K	Burlington, MA	>16	>32	>8	4	16	≤0.12	≤0.12
SHV-11, **SHV-12**, SHV-141, SHV-161, SHV-163, TEM-1	SHV-161 R43S **SHV-163 R43S, G238S, R292Q**	SHV 238S + 240K SHV WT (238G + 240E)	Burlington, MA	>16	>32	>8	>16	>64	0.25	≤0.12

[a] Enzymes with confirmed ESBL profiles are in bold. New variants are underlined.

[b] Putative ESBL SHV variants are in bold, and alterations that might be responsible for the extended profile are underlined.

[c] Only one SHV allele was carried by this isolate, and the protein sequence showed a mutation (V75 M) compared to SHV-11.

TABLE 4 Isolates displaying discrepancies ($n = 34$ isolates) between reference PCR and sequencing results and Check-MDR CT101 results[a]

				Check-MDR CT101 kit result														
Organism	City	State	PCR and sequencing results	CTX-M group 1	CTX-M group 2	CTX-M groups 8 + 25	CTX-M group 9	SHV ESBL	SHV WT	TEM ESBL	TEM WT	ACC	ACT/ MIR	CMYII	DHA	FOX	KPC	NDM-1
E. coli	Rochester	NY	CTX-M-15 + OXA-1/30	Pos														
	Lexington	KY	CTX-M-15 + OXA-1/30	Pos														
	New York	NY	CTX-M-15 + OXA-1/30	Pos														
	Aurora	CO	CTX-M-15 + OXA-1/30	Pos														
	New York	NY	CTX-M-15 + OXA-1/30	Pos														
	New Brunswick	NJ	CTX-M-15 + OXA-1/30	Pos														
	New York	NY	CTX-M-15 + OXA-1/30	Pos														
	Charlottesville	VA	CTX-M-15 + OXA-1/30	Pos														
	Detroit	MI	CTX-M-15 + OXA-1/30	Pos														
	Detroit	MI	CTX-M-15 + OXA-1/30	Pos														
	Rochester	NY	CTX-M-15 + OXA-1/30	Pos														
	Detroit	MI	CTX-M-15 + OXA-1/30	Pos														
	Charlottesville	VA	CTX-M-15 + OXA-1/30	Pos														
	Omaha	NE	CTX-M-15 + OXA-1/30 + PSE-like	Pos														
	New York	NY	CTX-M-15 + OXA-1/30 + TEM-1	Pos							Pos							
	Des Moines	IA	CTX-M-15 + OXA-1/30 + TEM-1	Pos							Pos							
	Akron	OH	CTX-M-15 + OXA-1/30 + TEM-1	Pos							Pos							
	Detroit	MI	CTX-M-15 + OXA-1/30 + TEM-1	Pos							Pos							
	Detroit	MI	CTX-M-15 + OXA-1/30 + TEM-1	Pos							Pos							
	Detroit	MI	CTX-M-15 + OXA-1/30 + TEM-1	Pos							Pos							
	New York	NY	CTX-M-27 + OXA-1/30				Pos											
	Charlottesville	VA	OXA-9 + SHV-7 + TEM-1					Pos			Pos							
	Louisville	KY	CTX-M-15	Pos							**Pos**							
	Pensacola	FL	CTX-M-15	Pos							**Pos**							
K. oxytoca	New Brunswick	NJ	OXY-2-11															
	New York	NY	OXY-2-11															
K. pneumoniae	New York	NY	CTX-M-15 + KPC-3 + OXA-1/30 + SHV-11 + TEM-1	Pos					Pos		Pos						Pos	
	New York	NY	CTX-M-15 + OXA-1/30 + SHV-1	Pos					Pos									
	Indianapolis	IN	CTX-M-15 + OXA-1/30 + SHV-1 + TEM-1	Pos					Pos		Pos							
	New York	NY	CTX-M-15 + OXA-1/30 + SHV-11 + TEM-1	Pos					Pos		Pos							
	Akron	OH	CTX-M-15 + OXA-1/30 + SHV-11 + TEM-1	Pos					Pos		Pos							
	Detroit	MI	CTX-M-15 + SHV-11	Pos					Pos		**Pos**							
	Lexington	KY	CTX-M-15 + OXA-1/30 + SHV-11 + **TEM-1**	Pos					Pos									
	Louisville	KY	FOX-5 + PSE-like + SHV-11						Pos							Pos		
	New York	NY	OXA-1/30 + SHV-7 + TEM-1					Pos	Pos		Pos							
	New York	NY	OXA-2 + SHV-1 + TEM-1					Pos	Pos		Pos							

[a] Underlined results were tests not included in the Check-MDR, and bold and underlined are tests included in the microarray showing discrepancies with reference methods. Pos, Positive.

presence was in agreement with reference methods. OXA-1/30, which is very prevalent in U.S. isolates, was not detected by the commercial microarray; however, in almost all isolates the gene encoding this enzyme was detected along with $bla_{CTX\text{-}M\text{-}15}$, causing no misclassification of isolates.

The presence of broad-spectrum and ESBL SHV enzymes in the *K. pneumoniae* strains carrying multiple alleles of this gene was detected by Check-MDR CT101, and the results of hybridizations with probes targeting 238G and 240E (broad spectrum or WT [kit nomenclature]) were noted in the raw data file; however, the software reported mutation 238S and/or 240K in all strains, categorizing those isolates as ESBL producers (Table 3).

One *K. pneumoniae* isolate displaying susceptible MICs to carbapenems (0.25 μg/ml for ertapenem and doripenem and 0.5 μg/ml for imipenem and meropenem) that was not initially tested by PCR for carbapenemase-encoding genes was positive for the KPC probe in the Check-MDR CT101. Results were confirmed by reference methods, and this isolate carried genes encoding KPC-3, SHV-11, and TEM-1.

The majority of the reports establishing the prevalence of β-lactamases and types of β-lactamases in U.S. institutions are performed within hospitals and/or specific subsets (4, 5, 16, 17). Studies quantitating the prevalence of β-lactamase types in comprehensive U.S. collections are very limited, and our findings clearly demonstrate that the diversity of β-lactamases has been relatively unrecognized in this geographic region; periodic surveillance of these enzyme-mediated resistances still seems to be a prudent practice.

Among ESBLs, CTX-M enzymes with or without other β-lactamases were very prevalent, and as in other regions of the world (2), CTX-M-15 was the most common unique ESBL type detected. Our collaborative investigations show that the majority of the CTX-M-15-producing *E. coli* strains from past surveys were ST131 (16), which represents a clinical threat due to its potential associated virulence (18). CTX-M-15-producing strains were noted in 20 hospitals located in 16 states, and the corresponding gene was dominantly detected in association with other enzymes, including KPC. CTX-M-15-producing strains have been reported in U.S. hospitals in the past decade (19–21). In the SENTRY Program study, the prevalence of isolates producing these CTX-M-15 enzymes among ESBL-phenotypic isolates was 16.0% in 2007 (22), compared to 33.8% in 2010. This increase in a short time period was noted previously in other countries and seems to be related to the fitness of the genetic structures carrying this particular ESBL (2).

Other CTX-M variants, including CTX-M-14, CMY-2, and SHV ESBL types, were also prevalent in this blood culture collection, and these observations are similar to the findings of Park et al. (4) among *E. coli* strains from three U.S. hospitals. In that study, all but one carbapenem-resistant strain produced KPC serine carbapenemases, showing that the increase in carbapenem resistance in that region was caused by the dissemination of this resistance determinant. The finding of a susceptible strain carrying bla_{KPC} detected by a commercial microarray is troublesome and should be further investigated. This observation could cause confusion in the clinical microbiology laboratory when reporting susceptibility results for this isolate, as the carbapenem MIC results were within the WT range.

A great variety of β-lactamases and combinations of these enzymes were detected among routine bacteremia *Enterobacteriaceae* strains collected during 2010 from U.S. hospitals. Most strains carrying the same enzyme combinations were from different bacterial species and institutions, indicating horizontal transfer of genetic structures instead of clonal spread. The presence of multiple β-lactamases in a single isolate has been recognized as an alarming fact since early detection of these enzymes (7). Although strains reported to produce two to four β-lactamases were commonly characterized, the detection of a single *K. pneumoniae* strain carrying up to eight β-lactamase-encoding genes, including a KPC carbapenemase and an inhibitor-resistant TEM variant (3), emphasizes the ability of these organisms to accumulate multiple resistance determinants. Among isolates from this study carrying multiple β-lactamases, *K. pneumoniae* strains seemed to have acquired a large variety of genes, including genes for various SHV variants that could be identified only with additional experiments. This finding has been reported previously (15, 23), whereas in this study, 14.1% of the *K. pneumoniae* strains had multiple SHV types and only 3 (4.2%) of these isolates carried a single β-lactamase-encoding gene.

An evaluation of β-lactamases is a costly and time-consuming task that requires considerable expertise. The use of Check-MDR CT101 might facilitate local epidemiological surveillance of a variety of β-lactamases (but not all); however, this microarray kit displayed high sensitivity and specificity compared to reference PCR and sequencing for detection of the most prevalent β-lactamases found among U.S. *E. coli* and *K. pneumoniae* isolates. Furthermore, this method was easy to use and seems suitable for many clinical microbiology laboratory applications.

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