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Contemporary (2010) Diversity of ß-lactamases among Enterobacteriaceae Collected from Bacteremias in USA Hospitals: Report from the SENTRY Antimicrobial Surveillance Program

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SENTRY ANTIMICROBIAL SURVEILLANCE

AMENDED ABSTRACT

Background: The dissemination of class A ß-lactamases (BL) with extended spectrum (ESBL) is a public health concern. In this study, we screened 214 Enterobacteriaceae (ENT) collected from bloodcultures in USA hospitals for ESBLs, plasmidic (p) AmpCs and narrow-spectrum BL.

Methods: 3,061 bloodculture isolates consecutively collected from 26 USA hospitals (20 states) during 2010 were susceptibility tested by CLSI methods. *Klebsiella* spp., *E. coli* (EC) and *P. mirabilis* (PM) strains were selected according the CLSI ESBL criteria for further evaluation. Species that hyperexpress chromosomal AmpC were selected using cefepime MIC values of ≥2 µg/mL. Isolates were tested by PCR and sequencing for genes encoding TEM, SHV, CTX-M, PER, VEB, GES, PSE, OXA-2, OXA-10, OXA-18/-45, OXA-1/30 and pAmpCs.

Results: Among 214 (7.0% overall) ENT isolates, 107 carried bla_{TEM} (mostly bla_{TEM-1}, but bla_{TEM-12} [EC] and $bla_{TEM-155}$ [PM] were also detected). SHV genes were detected among 89 strains. Most common types were SHV-11 and SHV-1 (9 variants total). Two isolates carried bla_{SHV-11}-like with additional alterations (L53Q/V75M). Evidence (double peaks in various loci) of two bla_{SHV} in the same strain was noted in 11 ENT. Genes encoding CTX-M-15, -14, -27 and -91 were detected in 67 (31.3%; 16 states), 8 (4.7%), 6 and 1 strain, respectively. pAmpCs were detected in 20 strains: 14 CMY (mostly CMY-2 in EC), 5 FOX-5 (3 *K. pneumoniae* [KPN], 1 PM, 1 *E. cloacae* [ECL]) and 1 DHA-1 (PM). bla_{PER-1} was observed in 3 strains (2 EC, 1 *P. vulgaris*) and bla_{PSE-1} in 5 strains (2 KPN, 1 EC, 1 ECL, 1 PM). 35 strains harbored bla_{OXA-30} (30 carried $bla_{CTX-M-15}$ and 13 had bla_{TEM-1}). 1 KPN carried genes encoding OXA-2, CTX-M-15, TEM-1 and SHV-1.

Conclusions: CTX-M-producers were previously reported (2007) in USA hospitals participating in the SENTRY Program (prevalence of 26.6%). Rates of strains carrying these enzymes seem to be increasing in USA hospitals (37.8% for 2010). Overall, a great variety of enzymes were detected and the number of isolates carrying multiple genes was elevated. A more limited number of strains carried pAmpC genes.

INTRODUCTION

Extended-spectrum ß-lactamases (ESBLs) are plasmid-mediated resistance determinants that confer elevated MIC values to penicillins, cephalosporins and aztreonam. The genes encoding these enzymes are encountered among members of the Enterobacteriaceae family and strains carrying ESBL-encoding genes are often resistant to other antimicrobial agents, such as trimethoprim/sulfamethoxazole, aminoglycosides and fluoroquinolones.

Plasmid-mediated AmpC enzymes (pAmpC) are Ambler class C cephalosporinases that are closely related in sequence to chromosomal AmpC enzymes from *Enterobacter cloacae*, *Morganella morganii*, *Citrobacter freundii* and *Halfnia alvei*. Most of these enzymes are constitutively expressed and produced at much higher levels than seen for basal AmpC cephalosporinases; however, some of the plasmid-encoded AmpC enzymes are inducible and might pose a higher clinical threat since during therapy the organisms can develop resistance to ß-lactam agents.

In the United States (USA), data concerning the prevalence of ESBLs and pAmpCs is very limited and in most cases, summarizes to a small number of strains collected in a single medical institution. In this study, we evaluated the presence of several ESBL, plasmid-mediated cephalosporinases and narrow-spectrum ß-lactamases among 214 Enterobacteriaceae strains collected from clinical bacteremias in 26 hospitals in the USA during 2010 as part of the SENTRY Antimicrobial Surveillance Program. Carbapenemases were also evaluated among those strains showing elevated carbapenem MIC values (imipenem or meropenem, ≥2 µg/mL).

MATERIALS AND METHODS

Bacterial isolates. A total of 3,061 Enterobacteriaceae bloodstream isolates were collected from 26 hospitals located in 20 USA states during the SENTRY Antimicrobial Surveillance Program (2010). Only one isolate per patient from documented bloodstream infections were included in the study. Species identification was confirmed by standard biochemical tests and the Vitek System (bioMerieux, Hazelwood, MO), when necessary.

Antimicrobial susceptibility testing. All isolates were susceptibility tested using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI, M07-A8). Categorical interpretations for all antimicrobials were those found in M100-S21 (2011) and quality control (QC) was performed using *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within specified ranges as published in CLSI documents (M100-S21).

Genotypic detection of ß-lactamases. *E. coli*, *Klebsiella* spp. and *Proteus mirabilis* strains were selected based on the CLSI criteria for ESBL epidemiological screening (M100-S21). Species that hyperexpress constitutive AmpC enzymes (*Enterobacter* spp., *Citrobacter* spp., etc.) were selected using cefepime MIC, ≥2 µg/mL. PCR screening was performed for bla_{TEM}, bla_{SHV} (singleplex reactions), bla_{CTX-M}, bla_{GES}, bla_{VEB}, bla_{PER}, bla_{PSE}, bla_{BEL}, and oxacillinases with ESBL spectrum (bla_{OXA-2}-, bla_{OXA-10}- and bla_{OXA-30}-group, bla_{OXA-18} and bla_{OXA-45}) in a combination of multiplex reactions. Additionally, $bla_{CMY-1-41}$, $bla_{CMY-43-44}$, bla_{CMY-49}, $bla_{FOX-1-7}$, $bla_{ACC-1-4}$, $bla_{ACT-1-7}$, $bla_{DHA-1-3}$, bla_{LAT-1}, $bla_{MIR-1-5}$, $bla_{MOM-1-7}$ were also amplified in a multiplex reaction.

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

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.ncbinlm.nih.gov/blast/).

RESULTS

- 214 (7.0% of the total) Enterobacteriaceae bloodculture isolates received from 26 USA hospitals were selected. These strains belonged to the following bacterial species: *E. coli* (94), *K. pneumoniae* (74), *Enterobacter cloacae* (25), *K. oxytoca* (10), *P. mirabilis* (6) and one of each *Citrobacter braakii*, *Enterobacter aerogenes*, *Enterobacter gergoviae*, *Providencia stuartii* and *Serratia marcescens*.
- The most prevalent enzymes detected by PCR and sequencing were CTX-M-15 (67 strains) and OXA-1/-30 (35 strains) and these enzymes, were often observed in the same strains (30 of 35). KPC-2, KPC-3, SHV-30, and CMY-2 were also prevalent (17, 12, 12 and 12 strains, respectively; Figure 1).
- Isolates carried at least 74 different enzyme arrays (two to five ß-lactamases) and are shown in Table 1.
- CTX-M enzymes were observed among 82 strains from 27 hospitals, 18 states. The most prevalent type was CTX-M-15 (67 strains), followed by CTX-M-14, -27 and -3 (eight, six and one strain, respectively).
- CTX-M-15 producing strains were noted in 20 hospitals located in 16 states. This gene was dominantly detected in association with other enzymes, including KPCs and a total 31 different ß-lactamase arrays carrying $bla_{CTX-M-15}$ were observed (Figure 2).
- SHV ESBLs were noted in 36 strains and the most common type was SHV-30 (13 strains), but SHV-2, SVH-5, SHV-7, SHV-12, SHV-27 were also detected. A total of 11 strains carried more than one type of SHV ß-lactamase, as noted by the presence of double peaks during sequencing analysis of the genes.
- Narrow spectrum ß-lactamases TEM-1 and SHV-11 were noted among 105 and 44 strains, respectively, alone or in combination with other enzymes. Two isolates had SHV-11-like enzymes with aminoacid substitutions L53Q and V75M. These strains were a *K. pneumoniae* and an *E. coli* from Virginia and Florida, respectively.
- TEM-12, TEM-155 (ESBLs) and SHV-63 (spectrum of activity unknown) were detected among *E. coli*, *P. mirabilis* and *K. pneumoniae*, respectively. Other ß-lactamase-encoding genes found included bla_{PER-1} (2 E. coli, 1 *P. vulgaris*), bla_{PSE}-like (five strains, four bacterial species) and bla_{OXA-2} (three strains, three bacterial species).
- pAmpCs were observed in 20 strains: 14 CMY (mostly CMY-2 in EC), five FOX-5 (three *K. pneumoniae*, one *P. mirabilis*, one *E. cloacae*) and one DHA-1 (*P. mirabilis*).

Table 1. ß-lactamase (enzymes) arrays detected among ESBL-phenotype-positive Enterobacteriaceae strains collected in USA hospitals (2010).

ß-lactamase arrays	No. of isolates	Percentage from total
TEM-1	11	5.1
CTX-M-15+OXA-1/-30+SHV-32	10	4.7
CTX-M-15+SHV-1+TEM-1	8	3.7
KPC-3+SHV-11+TEM-1	8	3.7
SHV-1	7	3.3
CTX-M-15+SHV-32	6	2.8
SHV-11	6	2.8
SHV-30	6	2.8
CTX-M-15+SHV-1	4	1.9
KPC-2+SHV-11+TEM-1	4	1.9
CMY-2+SHV-32	4	1.9
SHV-11+TEM-1	4	1.9
SHV-like+TEM-1	4	1.9
CTX-M-15+OXA-1/-30	3	1.4
CTX-M-15+OXA-1/-30+SHV-11	3	1.4
KPC-2+SHV-11	3	1.4
SHV-12+TEM-1	3	1.4
SHV-1+TEM-1	3	1.4
SHV-2	3	1.4
SHV-like	3	1.4
CTX-M-14+SHV-11+TEM-1	2	0.9
CTX-M-14+TEM-1	2	0.9
CTX-M-15+OXA-1/-30+SHV-1+TEM-1	2	0.9
CTX-M-15+OXA-1/-30+TEM-1	2	0.9
CTX-M-15+OXA-1/-30+SHV-like+TEM-1	2	0.9
CTX-M-15+SHV-11	2	0.9
CTX-M-15+TEM-1	2	0.9
CTX-M-15+SHV-like+TEM-1	2	0.9
CTX-M-27+SHV-11+TEM-1	2	0.9
CTX-M-27+SHV-1+TEM-1	2	0.9
CTX-M-27	2	0.9
FOX-5+PSE-like+SHV-11	2	0.9
KPC-2+CTX-M-15+SHV-1+TEM-1	2	0.9
KPC-2+SHV-1+TEM-1	2	0.9
KPC-2+TEM-1	2	0.9
OXA-2+SHV-7+TEM-1	2	0.9
PER-1+SHV-12+TEM-1	2	0.9
SHV-2+TEM-1	2	0.9
SHV-30+TEM-1	2	0.9
SHV-7+TEM-1	2	0.9
ACC-1+SHV-5	1	0.5
CTX-M-14	1	0.5
CTX-M-14+SHV-1+TEM-1	1	0.5
CTX-M-14+SHV-7+TEM-1	1	0.5
CTX-M-14+SHV-like+TEM-1	1	0.5
CTX-M-15-OXA-1/-30+PSE-like+SHV-7	1	0.5
CTX-M-15+OXA-1/-30+SHV-1	1	0.5
CTX-M-15+OXA-1/-30+SHV-11+TEM-1	1	0.5
CTX-M-15+OXA-1/-30+SHV-2	1	0.5
CTX-M-15+OXA-1/-30+SHV-30	1	0.5
CTX-M-15+OXA-1/-30+SHV-7+TEM-1	1	0.5
CTX-M-15+OXA-1/-30+SHV-like	1	0.5
CTX-M-15+OXA-2+SHV-1+TEM-1	1	0.5
CTX-M-15+PER-1+SHV-5+TEM-1	1	0.5
CTX-M-15+SHV-11+TEM-1	1	0.5
CTX-M-15+SHV-12+TEM-1	1	0.5
CTX-M-15+SHV-30	1	0.5
CTX-M-15+SHV-30+TEM-1	1	0.5
CTX-M-15+SHV-5+TEM-1	1	0.5
CTX-M-15+SHV-like	1	0.5
CTX-M-3	1	0.5
CTX-M-91+SHV-11+TEM-1	1	0.5
DHA-1+TEM-1	1	0.5
FOX-5+PSE-like+SHV-32	1	0.5
FOX-5+PSE-like+SHV-7	1	0.5
FOX-5+PSE-like+SHV-11	1	0.5
KPC-2+CTX-M-15+SHV-1	1	0.5
KPC-2+OXA-2	1	0.5
KPC-2	1	0.5
KPC-3+CTX-M-15+OXA-1/-30+SHV-11+TEM-1	1	0.5
KPC-3+CTX-M-15+SHV-11+TEM-1	1	0.5
KPC-3+TEM-1	1	0.5
CMY-2	1	0.5
CMY-2+SHV-11+TEM-1	1	0.5
CMY-2+SHV-1+TEM-1	1	0.5
CMY-2+SHV-30	1	0.5
CMY-2+TEM-1	1	0.5
CMY-2+SHV-33	1	0.5
CMY-2+SHV-5+TEM-1	1	0.5
CMY-2+SHV-like	1	0.5
CTX-M-15+CMY-like+SHV-1+TEM-1	1	0.5
CMY-like+LTEM-1	1	0.5
OXA-1/-30+SHV-11+TEM-1	1	0.5
OXA-1/-30	1	0.5
OXA-1/-30+TEM-1	1	0.5
OXA-1/-30+SHV-like+TEM-1	1	0.5
SHV-1+TEM-12	1	0.5
SHV-7	1	0.5
SHV-like+TEM-155	1	0.5

a. Includes: *Enterococcus avium* (four strains), *E. casseliflavus* (four strains), *E. durans* (four strains), *E. gallinarum* (five strains), and *E. raffinosus* (three strains).

Figure 1. Most common types of ESBL, pAmpC and carbapenemase enzymes detected in the ESBL-phenotype positive Enterobacteriaceae strains collected in USA hospitals in 2010. Strains were counted under the enzyme with the broadest hydrolytic profile and those could carry from 1 to 5 ß-lactamases.

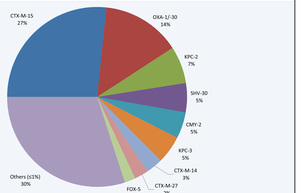

Figure 1. Distribution of CTX-M-15-producing isolates recovered from USA hospitals in 2010.

CONCLUSIONS

- A great variety of ß-lactamase arrays was detected among Enterobacteriaceae strains collected in USA hospitals. Most strains carrying the same enzyme arrays were from different bacterial species and institutions, indicating horizontal transfer of genetic structures instead of clonal spread.
- CTX-M-15-producing strains have been reported in the USA hospital in the past decade. In the SENTRY Program study, the prevalence of isolates producing these CTX-M-15 enzymes among ESBL-phenotypic isolates was 26.6% in 2007 compared to 37.8% 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.
- Few studies quantitating the prevalence of ß-lactamase types in comprehensive USA collections have been performed and surveillance of these enzymes still seems to be a prudent practice.

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