1688

ECCMID 2009

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Prevalence of Extended Spectrum ß-lactamases among Enterobacteriaceae Strains from the United States (USA; 2007) and Correlation with KPC Carbapenemases

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SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Objective: To determine the types and prevalence of ESBL enzymes among carbapenem-non-susceptible (CARB-NS) and carbapenem-susceptible (CARB-S) Enterobacteriaceae isolates with the correlation of ESBL-types with KPC production.

Methods: As part of the SENTRY Antimicrobial Surveillance Program, Enterobacteriaceae collected in USA medical centers (2007) with elevated cephalosporin MIC values (≥2 mg/L) by CLSI broth microdilution test were screened for the presence of ESBL-encoding genes. CTX-M, VEB, PER, OXA-2-like, OXA-10-like, TEM and SHV encoding genes were tested by PCR. Amplicons were sequenced on both strands. Carbapenemase genes (encoding KPC, IMP, VIM, NMC-A/IMI, SMEs and OXA-48) were screened in isolates showing MIC values at ≥2 mg/L for imipenem and/or meropenem. Statistical analyses were performed using Epi-Info 3.4.1.

Results: Among 2,844 Enterobacteriaceae, 287 strains (10% of the total) displayed elevated cephalosporins MIC values. Among those, 215 belonged to four species: *E. coli*, *K. pneumoniae* (KPN), *K. oxytoca* and *P. mirabilis*. The 72 remaining isolates were AmpC-producing species (dominantly *E. cloacae* [72%]). Fifty-two isolates showed elevated carbapenem MIC values associated with high MIC values for cephalosporins and KPC-production was detected in 45 strains (86%). At least 18 types of ß-lactamases were detected: CTX-M (4 variants; Table), SHV (9), TEM (2), OXA (2) and PER-like. Over 60% of the CARB-S isolates carried ESBL genes, while 48% of the CARB-NS strains carried these genes (p=0.04). However, a more limited variety of ß-lactamases was found among CARB-NS isolates when compared to CARB-S. TEM-1 and SHV-encoding genes were significantly more prevalent among KPC-producing isolates (77% for both; p<0.01) than in CARB-S strains (39% TEM-1 and 43% SHV). bla_{SHV-11} and bla_{SHV-12} were detected in 17 (37%) and 12 (34%) of the KPC-producers, respectively. All 35 KPN KPC-producing isolates detected in New York state sites also harbored TEM-1 and SHV-encoding genes, while 9 of the 12 KPN CARB-S carried both of these genes.

	ß-lactamase types (no. of isolates)																		
	CTX-M-				SHV-										TEM-	OXA-			
Organism groups (no.)	(15)	(14)	(2)	(3)	1	(5)	(7)	11	(12)	(27)	(30)	(31)	(40)		1	(10)	2	10	Other ß-lactmases
CARB-S (235)	34	22	1	1	19	10	10	20	26	2	10	4	1		93	1	16	6	(PER-like)
CARB-NS (51)[a]	-	1	-	-	4	1	-	18	12	-	3	-	1		45	-	2	2	-
KPC-producing isolates (45)	-	-	-	-	4	-	-	17	11	-	2	-	1		40	-	1	1	-

a. Include KPC-producing isolates.
*Enzyme variants with extended spectrum activity (ESBLs) are in parenthesis.

Conclusions: ESBL production was observed with the same frequency among CARB-S and CARB-NS isolates. However, KPC-encoding genes seemed to be more prevalent among SHV-producing isolates also carrying bla_{TEM-1}. SHV-11 was the most prevalent type, followed by SHV-12. Furthermore, these enzymes were found in the majority of CARB-S isolates from the same medical sites, suggesting that CARB-S strains carrying bla_{SHV} and bla_{TEM} have likely acquired KPC genes.

INTRODUCTION

Epidemiological surveys have demonstrated strikingly different ESBL production patterns among isolates from the USA compared to other countries. According to several surveillance studies, ESBL rates in USA medical centers in the beginning of this decade were approximately 5-10%. However, recent data demonstrated that in the intensive care units (ICU) of USA hospitals this numbers can be as high as 16%.

Various early USA surveys showed that TEM and SHV types of ESBLs were predominant, with fewer variants of both ESBL types identified. However, studies conducted in the late 90s indicating a growing diversity of SHV types, demonstrated that this enzyme had become more prevalent than TEM variants. Although these ESBLs are still commonly found among USA strains, the ESBL scenario recently changed with the reports of the dissemination of CTX-M-producing isolates in several USA hospitals. At least three CTX-M variants (CTX-M-15, CTX-M-14 and CTX-M-3) were identified among various Enterobacteriaceae species from hospitals located throughout the continental USA.

Isolates producing the KPC serine-carbapenemase have been detected in several USA hospitals and the presence of this carbapenemase is often associated with other resistance genes, including ESBL-encoding genes, such as bla_{SHV-12} and bla_{TEM-1}. In this study, we evaluated the presence of ESBL enzymes among 287 Enterobacteriaceae isolates from USA medical centers, 45 KPC-producing isolates.

MATERIALS AND METHODS

Bacterial isolates. A total of 2,844 Enterobacteriaceae isolates were collected from 26 USA hospitals during the SENTRY Antimicrobial Surveillance Program (2007). Only one isolate per patient from documented infections were included in the study. Isolates were collected from bloodstream, respiratory tract and skin and skin structures infections according to common protocols. Species identification was confirmed by standard biochemical tests and the Vitek System (bioMerieux, Hazelwood, Missouri, USA), when necessary.

Antimicrobial susceptibility testing and ESBL confirmation. All isolates were tested for antimicrobial susceptibility using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). Cation-adjusted Mueller-Hinton broth was used in validated panels manufactured by TREK Diagnostics (Cleveland, Ohio, USA). Categorical interpretations for all antimicrobials were found in M100-S19 and quality control (QC) was performed using *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within specified ranges as published in CLSI documents.

Isolates displaying the CLSI MIC criteria for ESBL production were confirmed with the clavulanate inhibition Etest (AB BIODISK, Solna, Sweden).

Genotypic detection of ESBLs and carbapenemase-encoding genes. Multiplex PCR approaches were used to detect ESBL and carbapenemase genes. Generic primers were used to detect PER, GES, VEB, CTX-M and oxacillinases (OXA-ESBL), TEM and SHV encoding genes.

Isolates showing reduced susceptibility to imipenem or meropenem (MIC, ≥2 mg/L) were tested for the presence of carbapenemase-encoding genes, including bla_{IMP}, bla_{VIM}, bla_{KPC}, bla_{SME}, bla_{GES} variants and for bla_{IMI}, bla_{NMC-A}, bla_{OXA-48}, combined in two amplification reactions.

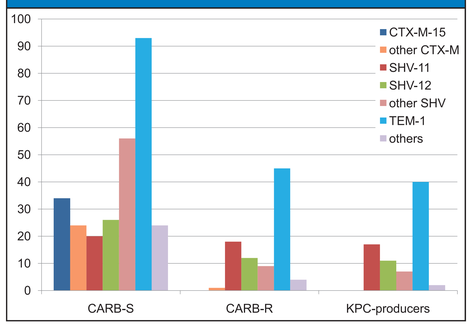
PCR amplicons were sequenced on both strands and the nucleotide sequences and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, WI). Sequences were compared to others available via internet sources (http://www.ncbinlm.nih.gov/blast/).

Statistical analysis. ESBL-production rates among different groups were calculated by χ^2 test using the Epi Info™ Version 3.4.1 software package (Centers for Disease Control and Prevention, Atlanta, GA, USA). p values < 0.05 were considered to be significant.

RESULTS

- A total of 287 strains displaying the CLSI criteria for ESBL production were tested. These isolates included 72 *Enterobacter* spp., *Citrobacter* spp., *Serratia* spp. and Indole-positive *Proteae* showing cefepime MIC values ≥2 mg/L.
- *K. pneumoniae* (106 strains), *E. coli* (77) and *E. cloacae* (53) were the most common species identified among the organisms evaluated.
- Fifty-two strains showed elevated carbapenem MIC results (≥2 mg/L for imipenem or meropenem) and among those 45 (86.5%) strains were found to carry KPC-encoding genes.
- The presence of ESBL genes was slightly dissimilar among carbapenem-susceptible and -resistant isolates (59.8% versus 48.4%, p=0.04). Overall, 18 ß-lactamase-encoding genes (ESBL and narrow-spectrum) were detected (**Table 1**).
- All genes observed among the KPC-producing strains were also detected among the carbapenem-susceptible organism group. However, a greater diversity of enzymes was observed among carbapenem-susceptible strains (18 and 10 types, respectively; **Figure 1**).

Figure 1. Distribution of ß-lactamase enzymes among carbapenem-susceptible (CARB-S), carbapenem-resistant (CARB-R) and KPC-producing isolates collected in the USA medical sites during the SENTRY Program (2007).



- bla_{TEM-1} was detected among all isolates groups, although its occurrence was considerably higher among the KPC-producing isolates (77.1%; p<0.01). Similar rates were noted for SHV-encoding genes, primarily bla_{SHV-11} and bla_{SHV-12} that were also significantly elevated among KPC-producing isolates (**Figure 1**).
- KPC was detected in 35 *K. pneumoniae* isolates from medical centers located in New York state, all harboring bla_{TEM-1} and bla_{SHV} genes. These ß-lactamase genes were also detected in 75.0% of carbapenem-susceptible *K. pneumoniae* from the same medical centers.

Table 1. Distribution of ß-lactamase enzymes among bacterial species and USA states.

ß-lactamase (no. of occurrences)	Enterobacteriaceae species	Medical Center location (state)
CTX-M-15 (34)	*E. coli*, *K. pneumoniae*	NY, TX, MI, WI, OH, WA
CTX-M-14 (23)	*E. coli*, *K. pneumoniae*, *P. mirabilis*	NJ, WA, MI, NY MA, KY, TX, HI, WI
CTX-M-2 (1)	*E. coli*	WA
CTX-M-3 (1)	*E. aerogenes*	HI
SHV-1 (23)	*K. pneumoniae*	NY, MA, WA, NE, KY, NJ, HI, MI, TX
SHV-5 (11)	*E. cloacae*, *K. pneumoniae*, esp, *Pantoea agglomerans*	OH, TX, MA, NJ
SHV-7 (10)	*E. cloacae*, *K. pneumoniae*, *S. marcescens*	KY, NY, MI
SHV-11 (38)	*E. cloacae*, *K. pneumoniae*	KY, WA, HI, MA, WI, MI, TX, NY, NJ
SHV-12 (38)	*E. cloacae*, *E. coli*, *K. oxytoca*, *K. pneumoniae*	TX, IN, VT, HI, NJ, MA, NY, WA, AR, VA
SHV-27 (2)	*K. pneumoniae*	WA
SHV-30 (13)	*E. cloacae*, *K. oxytoca*, *K. pneumoniae*, *S. marcescens*	KY, NJ, VA, AR, FL, WA
SHV-31 (4)	*K. pneumoniae*	NY, KY, MA
SHV-40 (2)	*K. oxytoca*, *K. pneumoniae*	AR
TEM-1 (138)	*E. cloacae*, *E. coli*, *K. oxytoca*, *K. pneumoniae*, *P. mirabilis*, *P. rettgeri*, *S. marcescens*, *Enterobacter* spp., *Klebsiella* spp.	MA, OH, MO, UT, MI, AR, NY, NE, WI, VT, WA, VA, KY, NJ, TX, HI, IA, IN
TEM-10 (1)	*S. marcescens*	WA
OXA-2 (18)	*E. cloacae*, *E. coli*, *S. marcescens*, *K. oxytoca*, *K. pneumoniae*, *Klebsiella* spp.	VA, WA, NY, FL, MA, AR, NJ, NY, IA, HI, KY, VA, TX
OXA-10 (8)	*E. coli*, *E. cloacae*, *K. oxytoca*, *K. pneumoniae*, *S. marcescens*,	NE, TX, VA, MI, MA, KY, WA
PER-5 (1)	*P. rettgeri*	TX

CONCLUSIONS

- SHV-11, one of the most prevalent ß-lactamase observed in the present study, was not noted by other surveys carried out from 1996 through 2003 in the USA. Interestingly, this ß-lactamase was found among carbapenem-susceptible and -resistant strains, including the KPC-producing subset.
- The same ß-lactamase types were detected among carbapenem-susceptible, carbapenem-resistant and KPC-producing strains, suggesting that bla_{KPC} has been acquired by carbapenem-susceptible strains circulating in these hospitals that already harboured other ß-lactamase genes.
- KPC and ESBL-encoding genes are often carried by plasmids that can also contain other resistant determinants. These structures may play an important role in the dissemination of these resistant genes.

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