High Prevalence of CTX-M-Producing Isolates and Emergence of KPC-encoding Genes in the Western United States

C2-669

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ICAAC 2009

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AMENDED ABSTRACT

Background: Prior studies show that β-lactamase-production alone was not an independent risk factor for treatment failure with β-lactams; however, failures were more influenced by pathogen MIC. We evaluated the β-lactamase content of isolates from an investigation of clinical outcomes of ertapenem non-susceptible and ESBL-producing Enterobacteriaceae.

Methods: Evaluated strains included 24 *E. coli* (EC), 13 *K. pneumoniae* (KPN), 1 *K. oxytoca* (KOX), 1 *E. cloacae* and 1 *P. mirabilis* (PM) collected from patients with various infections in Southern California hospitals. Susceptibility testing was by CLSI broth microdilution method. ESBL confirmation used clavulanate inhibition and carbapenemase screening applied the Modified Hodge test (MHT). Isolates were tested by PCR for ESBL-, carbapenemase- and plasmidic AmpC-encoding genes. Amplicons were sequenced.

Results: All isolates showed elevated cephalosporin MIC values (ceftriaxone and/or ceftazidime MIC, ≥8 µg/ml), and 24 (60.0%) displayed an ESBL confirmatory test. 19 CTX-M-producers were detected (47.5%; 16 EC, 1 KPN, 1 PM and 1 KOX). CTX-M-15 was most common, but CTX-M-14 was also observed. 32 (80.0%) and 13 (32.5%) strains harbored TEM and SHV-encoding genes, respectively. bla_{TEM-1} and bla_{SHV-11} (non-ESBL) were most prevalent. 5 (12.5%) KPN isolates were resistant to carbapenems (MIC, ≥8 µg/ml) and harbored bla_{KPC-3}. All KPC-producers harbored bla_{TEM-1}. Plasmidic AmpCs CMY-2 and DHA-1 were found in 4 EC and 2 KPN. OXA-2, SHV-12, TEM-17 and TEM-26 genes were also detected.

Conclusion: A high prevalence of CTX-M (nearly 50%) was observed among isolates collected as part of a clinical outcomes investigation. Additionally, we observed spread of KPC-producing isolates to the West Coast of the United States. ESBL-, KPC-production and ertapenem elevated MICs seemed to have minimal impact on clinical outcomes.

INTRODUCTION

Resistance against extended-spectrum cephalosporins among Enterobacteriaceae isolates is a serious clinical problem that usually leads to an increased use of carbapenems. According to surveillance studies, extended spectrum β-lactamase (ESBL) rates in United States (USA) medical centers in the beginning of this decade were approximately 5-10%; however, recent data demonstrated that in intensive care units (ICU) this numbers can be as high as 16%.

Epidemiological surveys in the USA demonstrated that TEM and SHV types of ESBLs were predominant, with fewer variants of both ESBL types identified. In the late 90s, 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) have been identified among various Enterobacteriaceae species from hospitals located throughout the continental USA.

Additionally, with the increasing use of carbapenems, 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 elements, such as bla_{SHV-12} and bla_{TEM-1}.

In the present study, we evaluated the presence of β-lactamase encoding genes among isolates recovered from an investigation of clinical outcomes of ertapenem non-susceptible and ESBL-producing Enterobacteriaceae in hospitalized patients from southern California, USA.

MATERIALS AND METHODS

Bacterial strains. Enterobacteriaceae strains potentially producing ESBLs were collected from various infections at 12 hospitals located throughout southern California (including Los Angeles, Orange and San Diego counties). Isolates were initially identified as ESBL-producers by the VITEK system and ertapenem MIC was initially determined by Etest® (AB bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions.

Antimicrobial susceptibility testing. All isolates were tested for antimicrobial susceptibility using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI). Cation-adjusted Mueller-Hinton broth was used in validated panels manufactured by TREK Diagnostics (Cleveland, Ohio, USA). Categorical interpretations for all antimicrobials were those 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.

Phenotypic detection of β-lactamases. ESBL production was confirmed with the clavulanate inhibition disk test according to CLSI guidelines. All isolates with reduced susceptibility to ertapenem (MIC, ≥1 µg/ml) were tested with the Modified Hodge test (MHT) using imipenem and meropenem disks.

Genotypic detection of β-lactamase-encoding genes. Multiplex PCR approaches were used to detect β-lactamase genes. Generic primers were used to detect PER, GES, VEB, CTX-M and oxacillinases (OXA-ESBL). Isolates 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. Plasmidic AmpC encoding genes were detected as described elsewhere. TEM and SHV were amplified separately using generic primers. 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/).

Molecular typing. Genomic DNA was prepared in agarose blocks, digested with Spe I (New England, Beverly, MA) and electrophoresis was performed on the CHEF-DR III apparatus (BioRad, Richmond, CA). Interpretation of the results was performed as previously described.

RESULTS

- A total of 40 Enterobacteriaceae strains were collected from patients with various infections in southern California hospitals between August 1, 2007 and March 31, 2009.
- All isolates demonstrated elevated MIC values (≥2 µg/ml) for ceftriaxone, ceftazidime and/or cefepime, and 24 strains (60.0%) displayed a positive ESBL confirmatory test.
- Strains carried at least one β-lactamase encoding gene and 30 Enterobacteriaceae possessed multiple β-lactamase genes (Table 1).
- bla_{CTX-M} was detected alone or with other β-lactamase genes in 19 strains (47.5%). $bla_{CTX-M-15}$ was the most common enzyme type detected in three bacterial species. $bla_{CTX-M-14}$ was detected in 5 isolates (4 *E. coli* and 1 *P. mirabilis*).
- Five strains harbored the same β-lactamase array that included bla_{KPC-3}. Two of these strains were collected from the same patient and all strains were collected from the same hospital.
- KPC-producers showed elevated MIC values for all carbapenems (imipenem, meropenem and ertapenem MIC values at ≥8 µg/ml). PFGE analysis showed that KPC-3-producing strains were genetically related.
- Five strains harbored plasmidic AmpC genes encoding CMY-2 and DHA-1 alone or with other β-lactamases (Table 1).
- Resistance rates against extended spectrum cephalosporins were elevated and isolates also showed elevated resistance rates to a fluoroquinolone (levofloxacin; 82.9%), tetracycline (63.4%) and trimethoprim/sulfamethoxazole (61.0%; Table 2).
- The majority of the strains were susceptible to amikacin, meropenem and tigecycline (75.6, 85.4 and 97.6% susceptible, respectively).

Table 1. Enterobacteriaceae isolates producing β-lactamases collected in southern California hospitals.

β-lactamases	Bacterial species (no. of isolates)
CMY-2	*E. coli* (1)
CMY-2, TEM-1	*E. coli* (1)
CTX-M-14	*E. coli* (1)
CTX-M-14, TEM-1	*E. coli* (3), *P. mirabilis* (1)
CTX-M-15	*E. coli* (2), *K. pneumoniae* (1)
CTX-M-15, TEM-1	*E. coli* (7), *K. oxytoca* (1)
CTX-M-15, TEM-26	*E. coli* (2)
CTX-M-15, DHA-1, TEM-26	*E. coli* (1)
DHA-1	*E. coli* (1)
DHA-1, SHV-1, TEM-1	*K. pneumoniae* (1)
DHA-1, SHV-11/-36, TEM-17	*K. pneumoniae* (1)
KPC-3, SHV-11/-36, TEM-1	*K. pneumoniae* (5)
OXA-2, TEM-1	*E. cloacae* (1)
SHV-1, TEM-1	*K. pneumoniae* (3)
SHV-11, TEM-1	*K. pneumoniae* (2)
SHV-12, TEM-1	*E. coli* (1)
TEM-1	*E. coli* (3)
TEM-26	*E. coli* (1)

Table 2. Comparative vitro activity of selected antimicrobial agents tested against Enterobacteriaceae[a] (40 strains).

Antimicrobial agent	MIC_{50}	MIC_{90}	% susceptible/ resistant[b]
Ceftriaxone	>32	>32	9.8 / 82.9
Ceftazidime	>16	>16	17.1 / 68.3
Cefepime	16	>16	47.5 / 50.0
Cefoxitin	>16	>16	22.0 / 65.9
Ertapenem	0.25	>8	73.2 / 19.5
Meropenem	≤0.12	>8	85.4 / 12.2
Piperacillin/tazobactam	64	>64	41.5 / 43.9
Amikacin	4	32	75.6 / 2.4
Gentamicin	>8	>8	43.9 / 51.2
Levofloxacin	>4	>4	12.2 / 82.9
Tetracycline	>8	>8	19.5 / 63.4
Tigecycline[c]	0.25	1	97.6 / 0.0
Trimethoprim/sulfamethoxazole	>2	>2	39.0 / 61.0

a. Includes: *Enterobacter cloacae* (1 strain), *Escherichia coli* (25 strains), *Klebsiella oxytoca* (1 strain), *Klebsiella pneumoniae* (13 strains), and *Proteus mirabilis* (1 strain).
b. Criteria as published by the CLSI [2009].
c. USA-FDA breakpoints were applied [Tygacil Product Insert, 2005].

CONCLUSIONS

- CTX-M enzymes were detected in nearly 50.0% of the evaluated strains. These enzymes are becoming more common in USA hospitals, similarly to other countries where these CTX-M variants are broadly disseminated.
- KPC-3-producing isolates were collected from four patients and were clonally related. Three of these patients were hospitalized in long term care facilities and were admitted to the hospital already presenting the infection caused by the KPC-3-producing *K. pneumoniae*.
- The variety of β-lactamase arrays in the strains evaluated during this study indicates a complex process of acquisition and dissemination of genes encoding these enzymes within this geographic area.

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