Occurrences of Extended Spectrum and Other ß-lactamases Among Enterobacteriaceae Isolated in USA Medical Centers: 10-Year Report from the SENTRY Antimicrobial Surveillance Program

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ABSTRACT

Background: Emergence of ESBLs has compromised activity of potent ß-lactams and aztreonam (ATM) as empiric treatments of ENT infections. Understanding in vitro ESBL detection and ENT breakpoint criteria is critical to assessing isolate susceptibility (S) and initiation of interventions. Prevalence/types of ß-lactamases (BL) in SENTRY Program participant sites were tabulated (1997-2006).

Methods: Enterobacteriaceae (ENT) isolates collected from >25 USA centers/year were S tested (CLSI) including *E. coli* (EC; 13,780), *Klebsiella* spp. (KSP; 7,162) and *P. mirabilis* (PM; 1,224) with ESBL-screening criteria (MIC, ≥2 µg/ml for ATM or ceftriaxone or ceftazidime). Confirming tests used ESBL Etests or clavulanate (CLA) disk methods. Cefepime (FEP) MIC at ≥4 µg/ml was the ESBL screen criterion for *Enterobacter* spp. (ESP; 3,147), *C. freundii* (CF; 488) and *S. marcescens*. (SM; 1,537) with confirmation by FEP + CLA. PCR was also performed for serine carbapenemases (SC) and mobile Amp-C enzymes (MAC).

Results: EC ESBL rates increased from <1% to 3.1% (endemic clonality) while CMY-2, FOX-5 and DHA-1 rates remained constant (3.0%) and KPC-2 or -3 BLs emerged in 2002 (New York area; NY). KSP ESBLs expanded into 50% more hospitals and rates rose from 6.1 to 8.5% with ESBL phenotypes increasing to 15.6% driven by endemic KPC (4 sites) occurrences. PM strains rarely have ESBL (+) tests at 1.0%, occurring in centers with high ESBL/KPC rates in KSP. ESBL rates in CF, ESP and SM were 0.2, 1.6 and 0.3%, respectively. Recently, SCs have been documented in CF (2002), ESP (2003) and SM (2004) all in NY. ESBL strains were often resistant to fluoroquinolones and aminoglycosides, but remained S to polymyxins and tigecycline.

Conclusions: Positive ESBL-screening criteria were most often confirmed for KSP (54.5-82.4%) and less for EC and other spp. (10.0-60.0%). ESBL prevalence has increased in all spp. during the last decade, primarily due to inter- and intra-hospital clonal spread. Plasmidic movement of ESBL, MAC and SC between ENT species has markedly complicated detection criteria and empiric therapies.

INTRODUCTION

The utility of advanced spectrum ß-lactams in clinical practice (treatment of Enterobacteriaceae infections) has been compromised by the rapid evolution and dissemination of extended-spectrum ß-lactamases (ESBL). While more than 600 distinct ß-lactamases have been described, the Bush group 2be of ESBLs have proliferated and constitute the largest subset of all such enzymes. ESBLs are usually plasmid-mediated enzymes that variably hydrolyze oxyimino-cephalosporins and monobactams. However, they constitute a heterogeneous molecular cluster (20 to >90% identity) with great diversity in substrate affinity and susceptibility profiles. Clinical failures during cephalosporin therapy of infections caused by ESBL-producing strains are well documented, even among isolates testing susceptible (MIC, ≤8 µg/ml for "third- and fourth-generation" agents).

Current Clinical and Laboratory Standards Institute (CLSI) documents recommend the performance of confirmatory ESBL testing (clavulanate inhibition) of any enteric isolate (*Escherichia coli*, *Klebsiella pneumoniae*, *K. oxytoca* or *Proteus mirabilis*) with MIC results of ≥2 µg/ml to ceftazidime and/or ceftriaxone (cefotaxime) and/or aztreonam. Given the widespread occurrence and increasing prevalence of ESBLs, considerable discussion has ensued as to the appropriateness of current cephalosporin breakpoints (too high) and whether the laboratory testing community would be better served by having such breakpoints adjusted downward based upon contemporary pharmacokinetic/pharmacodynamic (PK/PD) and clinical data. Published studies have identified breakpoints that are superior predictors of successful treatment outcomes, and obviate the need for expensive, follow-up confirmatory ESBL testing.

The SENTRY Antimicrobial Surveillance Program has monitored trends in the spread of antimicrobial resistances from Europe, the Americas and the Asia-Pacific region since 1997. We describe the 10-year trend among Enterobacteriaceae isolates (1997-2006) from the United States (USA) displaying ESBL phenotype characteristics with selected determinations of enzyme type and the documented emergence of epidemic serine carbapenemases.

MATERIALS AND METHODS

Bacterial isolates: A total of 29,516 Enterobacteriaceae isolates were collected from 50 medical centers in 30 states as part of the USA SENTRY Antimicrobial Surveillance Program (1997-2006). Consecutive, non-duplicate strains were collected from bloodstream, respiratory tract, urinary tract and skin/soft tissue infections. All isolates were identified by the participant laboratories and confirmed by the monitoring facilities (JMI Laboratories, North Liberty, IA).

Susceptibility testing: All isolates were susceptibility tested by the CLSI (M7-A7) reference broth microdilution method using validated panels with cation-adjusted Mueller-Hinton broth (TREK Diagnostics; Cleveland, OH). Categorical interpretations of susceptibility to tested antimicrobials were those published by the CLSI (M100-S17), where available. Breakpoints for Enterobacteriaceae, when testing tigecycline, were those of the USA-FDA (≤2 / ≥8 µg/ml for susceptible/resistant). Concurrent quality control (QC) testing was performed using *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 among others; all QC results were within published CLSI ranges.

ESBL confirmatory tests: *E. coli*, *K. pneumoniae, K. oxytoca* and *P. mirabilis* isolates meeting ESBL-screening criteria (CLSI; MIC values ≥2 µg/ml for aztreonam and/or ceftriaxone and/or ceftazidime) were further processed by confirmatory tests using ESBL Etests (AB BIODISK, Solna, Sweden) and/or the published clavulanate disk approximation method. A cefepime MIC at ≥4 µg/ml was the ESBL screening criterion utilized for *Enterobacter* spp., *Citrobacter* spp. and *Serratia* spp. with confirmation performed using cefepime and clavulanic acid-containing Etests. Isolates with an ESBL phenotype, but a negative confirmatory test, were considered potential Amp-C producers and screened for production of Amp-C by the 3D screening test, using cefoxitin as a substrate. *K. pneumoniae* ATCC 700603 was used as a QC strain for all ESBL studies.

Molecular studies: Clonality was assessed for strains demonstrating ESBL-like resistance or highly similar antibiograms by molecular procedures utilizing the Riboprinter Microbial Characterization System® (Qualicon, Wilmington, DE) followed by further epidemiologic discrimination of common ribotypes using CHEF-DRII pulsed-field gel electrophoresis (PFGE; BioRad Laboratories, Hercules, CA).

In addition, all isolates with elevated carbapenem MIC values (≥2 µg/ml) and a negative metallo-ß-lactamase test, were screened for IMI-, KPC-, NMC-A and SME-type serine carbapenemases using specific primers. ESBL *bla* genes were amplified from selected strains using the following oligonucleotide primers: bla_{TEM} *(5'-ATAAAATTCTTGAAGACGAAA-3', 5'-GACAGTTACCAATGCTTAATCA-3')*, bla_{SHV} *(5'-TCGGGCCGCGTAGGCATGAT-3'), 5'-AGCAGGGC-GACAATCCCGCG-3')*, bla_{CTX-M} *(5'-TTAATGATGACT-CAGAGCATTC-3', 5'-GATACCTCGCTCCATTTATTG-3')*, and bla_{OXA} (5'-TTCAAGCCAAAGCACGATAG-3', 5'-TCCGAGTTGACTGCGGGTTG-3'). Those *E.coli* isolates (2005-2006) with a positive 3D Amp-C test were screened for nine different families of acquired Amp-C genes (MOX-1, -2; CMY-1 to -11; LAT-1 to -4; BIL-1; DHA-1, -2; ACC; MIR-1; ACT-1; FOX-1 to -5) by a multiplex PCR screening procedure described previously. Tentative enzyme type designations were assigned based on the size of the PCR amplicon and further confirmed by amplification with bla_{Amp-C} specific primers followed by sequencing. The PCR products were processed using the QIAGEN PCR purification kit. Sequencing was performed using the Sanger-based dideoxy sequencing strategy involving the incorporation of fluorescent-dye-labeled terminators into the sequencing reaction products. Sequences obtained were compared to the publicly available sequences via NCBI BLAST search to determine the type of ß-lactamase.

RESULTS

- Broad-spectrum ß-lactams (ceftazidime, ceftriaxone, cefepime, aztreonam, piperacillin/tazobactam, imipenem), fluoroquinolones (ciprofloxacin), aminoglycosides (tobramycin) and newer agents (tigecycline) have exhibited high-level coverage (>90%) of Enterobacteriaceae in the USA over the last decade (Table 1).

Table 1. Activity of select antimicrobial agents tested against seven groups of Enterobacteriaceae (29,516 strains) isolated in the United States (SENTRY Antimicrobial Surveillance Program, 1997 – 2006).

Organism/ antimicrobial agents (no.tested)	MIC (µg/ml) 50%	MIC (µg/ml) 90%	% by category[a] Susceptible	% by category[a] Resistant (ESBL-phenotype)
E. coli (13,780)				
Ceftazidime	≤1	≤1	98.1	1.3 (3.3)[b]
Ceftriaxone	≤0.25	≤0.25	98.4	0.8 (2.5)
Cefepime	≤0.12	≤0.12	99.4	0.5
Aztreonam	≤0.12	0.25	98.5	1.0 (3.5)
Piperacillin/tazobactam	2	4	96.7	1.5
Imipenem	≤0.5	≤0.5	100.0	0.0
Ciprofloxacin	≤0.25	≤0.25	91.1	8.7
Tobramycin	1	2	95.9	2.3
Tigecycline	0.12	0.25	100.0	0.0
K. pneumoniae (5,990)				
Ceftazidime	≤1	2	92.2	7.1 (8.8)
Ceftriaxone	≤0.25	0.5	94.3	2.8 (8.5)
Cefepime	≤0.12	0.25	97.6	1.7
Aztreonam	≤0.12	0.5	92.9	6.5 (8.5)
Piperacillin/tazobactam	2	16	93.1	4.4
Imipenem	≤0.5	≤0.5	98.9	0.9
Ciprofloxacin	≤0.25	≤0.5	92.3	6.4
Tobramycin	0.5	1	92.1	6.2
Tigecycline	0.5	1	98.7	0.0
K. oxytoca (1,104)				
Ceftazidime	≤1	2	95.7	3.9 (14.6)
Ceftriaxone	≤0.25	4	93.8	1.4 (12.3)
Cefepime	≤0.12	0.5	99.4	0.4
Aztreonam	≤0.12	8	90.2	8.2 (14.7)
Piperacillin/tazobactam	2	64	88.1	9.7
Imipenem	≤0.5	≤0.5	99.8	0.1
Ciprofloxacin	≤0.25	≤0.25	93.6	5.3
Tobramycin	0.5	1	94.3	3.6
Tigecycline	0.25	1	100.0	0.0
P. mirabilis (1,657)				
Ceftazidime	≤1	≤1	99.2	0.6 (2.1)
Ceftriaxone	≤0.25	≤0.25	99.4	0.4 (1.3)
Cefepime	≤0.12	≤0.12	99.6	0.3
Aztreonam	≤0.12	≤0.12	99.0	0.9 (1.5)
Piperacillin/tazobactam	≤0.5	1	99.4	0.3
Imipenem	1	2	99.6	0.0
Ciprofloxacin	≤0.25	>2	84.1	12.3
Tobramycin	1	4	95.4	2.5
Tigecycline	2	4	68.6	3.0
Citrobacter spp. (941)				
Ceftazidime	≤1	>16	84.3	13.8
Ceftriaxone	≤0.25	32	86.1	6.3
Cefepime	≤0.12	0.5	99.5	0.3 (0.9)
Aztreonam	≤0.12	>16	85.2	11.0
Piperacillin/tazobactam	2	32	87.8	4.7
Imipenem	≤0.5	1	99.9	0.1
Ciprofloxacin	≤0.25	0.5	92.3	5.2
Tobramycin	0.5	2	93.4	5.3
Tigecycline	0.25	0.5	99.1	0.0
Enterobacter spp. (4,086)				
Ceftazidime	≤1	>16	77.4	19.0
Ceftriaxone	≤0.25	>32	80.5	10.7
Cefepime	≤0.12	2	98.5	0.8 (7.2)
Aztreonam	≤0.12	0.25	79.4	15.6
Piperacillin/tazobactam	2	64	81.4	7.6
Imipenem	≤0.5	1	99.7	0.2
Ciprofloxacin	≤0.25	0.5	92.9	5.1
Tobramycin	0.5	2	92.6	5.4
Tigecycline	0.5	1	97.0	0.3
Serratia spp. (1,958)				
Ceftazidime	≤1	2	96.9	2.1
Ceftriaxone	≤0.25	2	95.5	1.4
Cefepime	≤0.12	0.25	99.2	0.4 (2.3)
Aztreonam	≤0.12	1	96.7	2.6
Piperacillin/tazobactam	2	8	95.1	1.5
Imipenem	≤0.5	2	99.6	0.3
Ciprofloxacin	≤0.25	0.5	94.8	3.3
Tobramycin	2	4	91.3	2.6
Tigecycline	1	2	97.0	0.7

a. CLSI interpretive criteria for all agents except tigecycline, where US-FDA package insert breakpoints were applied.
b. Percentage in parenthesis indicates the proportion of strains having an ESBL phenotype (CLSI, 2007).

- Ciprofloxacin resistance was greatest (12.3%) among *P. mirabilis*, and the ß-lactams were least active against *Citrobacter* spp. and *Enterobacter* spp.

- Analysis of ß-lactamase-mediated resistance trends (Table 2) showed:
  - ESBL expansion among monitored medical centers
  - increasing ESBL phenotypic profiles
  - increasing proportion of confirmed ESBL-producing strains (*E. coli* only)
  - escalating epidemic clonality within many medical centers
  - increasing numbers of non-ESBL mechanisms that produce an ESBL phenotype pattern [plasmidic Amp C and KPC enzymes; KPC-3 producing strain (*E. coli*)] was detected as early as 1998.

- ESBL and KPC-type serine carbapenemases have been detected among ESBL phenotypes in *C. freundii*, *Enterobacter* spp. and *Serratia* spp. (Table 3). ESBL rates remained low in non-*E. coli* and-*Klebsiella* spp. isolates (0.2-1.6% over all years monitored).

Table 2. Trends by year in ESBL rates among *E. coli* (13,780 strains) and *Klebsiella* spp. (7,162 strains) isolated by the USA SENTRY Program (1997-2006).

Organism (no. tested)/ years sampled	ESBL% Phenotype	ESBL% Confirmed	ESBL% Net rate	% of sites	No. epidemic clones
E. coli (13,780)					
1997-1998	3.6	18.1	0.7	25.0	3
1999-2000	2.9	14.7	0.4	15.7	1
2001-2002	5.0	25.2	1.3	30.0	6[a]
2003-2004	4.0	55.0	2.2	62.5	13[b]
2005-2006	6.3	50.9	3.2	64.9	15[c]
Klebsiella spp. (7,162)					
1997-1998	9.1	61.4	5.6	46.7	14[d]
1999-2000	6.4	60.0	3.8	71.8	11
2001-2002	11.8	72.3	8.5	55.3	14[e]
2003-2004	11.6	67.2	7.8	86.1	22[f]
2005-2006	16.9	54.6	9.2	77.5	22[g]

a. Epidemic clones included a OXA-1 + SHV-5 and the initial appearance of a KPC-3 serine carbapenem in New York (1998).
b. Non - ESBL enzymes were typed (CMY-2 [13], FOX-5 [3], DHA-1 [1], other Amp C [8] and KPC-3 [1; same site as 1998 and 2002 occurrences]).
c. Included 2 clones of KPC-2 in different sites involving bloodstream infections.
d. Documented OXA-2 + TEM-1 + SHV-5 epidemic at site 015.
e. OXA-2 + SHV-7 epidemic at site 015, TEM-1 + SHV-5 at site 004, and KPC-2 emerges in epidemics at several sites.
f. KPC-2 and -3 epidemic clones expand beyond New York area.
g. Continued expansion of KPC-2 and -3 epidemic in background of persisting ESBL and plasmidic Amp C clones.

Table 3. ESBL-producing strains among *C. freundi* (488 strains), *Enterobacter* spp. (3,147 strains), *P. mirabilis* (1,224 strains) and *S. marcescens* (1,537 strains) in the USA SENTRY Program (1997-2006).

Organism (no. tested)	ESBL% Phenotype	ESBL% Confirmed	ESBL% Net rate	Comments
C. freundi (488)	1.2	20.0	0.2	KPC-3 found in 2002; ESBLs in 5 medical centers
Enterobacter spp. (3,147)	3.3	47.6	1.6	Clonal KPC enzymes noted from 2003; ESBLs in some centers (17 total)
P. mirabilis (1,224)	2.7	35.7	1.0	Low rate of ESBL
Serratia spp. (1,537)	0.8	44.4	0.4	KPC-3 and ESBLs detected

CONCLUSIONS

- ESBL-producing strains have increased among *E.coli* (0.4 to 3.2%) due to clonal expansion into and between medical centers in the SENTRY Program (1997-2006). Also ESBL isolates increased from 3.8 to 9.2% among *Klebsiella* spp.

- Overall, ESBL phenotypic resistance increased at a greater rate, dominantly driven by emerging occurrences of plasmidic Amp C enzymes (CMY-2, FOX-5, etc.) and serine carbapenemases (usually KPC-types). SENTRY Program ESBL phenotypic resistance rates are currently 6.3 and 16.9% for *E.coli* and *Klebsiella* spp. isolates (2006).

- Genetic dissemination from endemic/epidemic reservoirs (*E. coli* and *Klebsiella* spp.) to other Enterobacteriaceae has been documented for ESBL and KPC enzyme encoding genes for *P. mirabilis* (ESBL), *C. freundii* (ESBL and KPC-3), *Enterobacter* spp. (ESBL, and KPC-2 and -3) and *Serratia* spp. (ESBL, and KPC-2 and -3); see Table 3.

- SENTRY Program results from ESBL resistance monitoring over the last decade indicates significant increases in the occurrence of ESBL, plasmidic Amp C and serine carbapenemases among Enterobacteriaceae species; however, the rates remain well below those observed in Europe, Latin America and many countries in the Asia-Pacific region. The greatest concern appears to be the epidemic spread of KPC enzyme-producing strains with resistance to essentially all marketed ß-lactams.

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