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Prevalence of Confirmed ESBL Production among European Enterobacteriaceae: a Ten Year Report from the SENTRY Antimicrobial Surveillance Program

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SENTRY ANTIMICROBIAL SURVEILLANCE

AMENDED ABSTRACT*

Objectives: To describe the 10-year trend among European (EUR) Enterobacteriaceae (ENT) isolates displaying phenotypic characteristics of extended-spectrum beta-lactamases (ESBL). The global emergence of ESBLs has compromised the activity of penicillins, third- and fourth-generation cephems and aztreonam (ATM) as empiric agents when treating infections caused by ENT. Understanding of recommended ESBL detection criteria is critical for the assessment of isolate susceptibility (S) and initiation of necessary infection control measures.
Methods: A total of 30,137 ENT isolates collected from 44 medical centers in 18 EUR countries including Russia were S tested as part of the SENTRY Antimicrobial Surveillance Program (1997-2006). *E. coli* (EC), *K. pneumoniae* (KPN), *K. oxytoca* (KOX) and *P. mirabilis* (PM) isolates meeting ESBL-screening criteria (CLSI; MIC values ≥2 mg/L for ATM or ceftriaxone or ceftazidime) were confirmed using ESBL Etests (AB BIODISK) or the clavulanate (CLA) disk approximation method. A cefepime (FEP) MIC at ≥4 mg/L was the ESBL screening criterion for *Enterobacter* spp. (ESP), *Citrobacter* spp. (CSP) and *Serratia* spp. (SER) with confirmation performed using FEP with CLA.
Results: Among the 26,859 ENT isolates examined for ESBL production, 2,963 (11.0%) qualified as potential ESBL producers. A subset of 1,780 were tested using a confirmatory method with 1,358 (76.3%) being positive. Confirmation of screening criteria occurred most frequently (>70%) in KPN, KOX, EC, CSP, SER and least often (<70%) when testing ESP and PM. Among EC and KPN, ESBL-screen positivity rates have increased from 1997-1999 to 2004-2006 (4.7-7.0% and 23.0-27.3%, respectively). Occurrence of ESBL screen positive isolates that did not confirm with CLA inhibition may be attributable to other recognized R mechanisms. Confirmed ESBL production was often associated with R to fluoroquinolones and aminoglycosides. S to carbapenems, tigecycline, and polymyxin B was retained among ESBL-confirmed isolates.

Organism (no. tested)	ESBL Screening Criteria Results: No. Detected	ESBL Screening Criteria Results: % Positive	ESBL Confirmatory Test Results: No. Tested	ESBL Confirmatory Test Results: No. (%) Positive
EC (15,027)	1,013	6.7	654	485 (74.2)
KPN (3,881)	1,045	26.9	728	652 (89.6)
KOX (1,121)	215	19.2	96	80 (83.3)
PM (1,590)	172	10.8	70	40 (57.1)
ESP (3,369)	422	12.5[a]	190	68 (35.8)
CSP (683)	31	4.5[a]	10	8 (80.0)
SER (1,188)	65	5.5[a]	32	25 (78.1)
Total (26,859)	2,963	11.0	1,780	1,358 (76.3)

a. ESBL phenotype screening criterion of FEP ≥4 mg/L.

Conclusions: Among tested EUR ENT, ESBL-screening criteria were most often confirmed among KPN (89.6%) and KOX (83.3%) and less so for EC (74.2%) and other species. ESBL prevalence varied widely, but has increased very slightly during this 10-year study, primarily in KPN and EC. Plasmidic movement of ESBLs between ENT species is a likely product of the high prevalence within predominant species, a worrisome development warranting continued longitudinal monitoring.

* Updated to reflect changes to numbers of isolates.

INTRODUCTION

The utility of advanced generation ß-lactams in clinical medicine has been compromised by the rapid evolution and dissemination of extended-spectrum ß-lactamase (ESBL) enzymes. While more than 600 distinct ß-lactamases have been described, the Bush 2be group of ESBLs have proliferated and constitute the largest subset of all such enzymes. ESBLs are plasmid-mediated enzymes that variably hydrolyze oxyimino-cephalosporins and monobactams. They constitute a heterogeneous molecular cluster (20 to >90% identity) with great diversity in substrate preferences and susceptibility profiles. Clinical failures during cephalosporin therapy of infections caused by ESBL-producing strains are well-known, even among isolates testing susceptible (MIC, ≤8 mg/L 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 isolate (*Escherichia coli*, *Klebsiella pneumoniae*, *K. oxytoca* or *Proteus mirabilis*) with MIC results of ≥2 mg/L to ceftazidime, ceftriaxone 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 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 1998. Here we describe the 10-year trend among European Enterobacteriaceae isolates (1997-2006) displaying ESBL phenotype characteristics.

MATERIALS AND METHODS

Bacterial isolates: A total of 30,137 Enterobacteriaceae isolates were collected from 44 medical centers in 18 European countries as part of the 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.

Susceptibility testing: All isolates were susceptibility tested by the CLSI (M7-A7) reference broth microdilution method using validated dry-form panels with cation-adjusted Mueller-Hinton broth (TREK Diagnostics, Cleveland, USA).

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 mg/L 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 mg/L for aztreonam or ceftriaxone or ceftazidime) were subjected to confirmatory tests using ESBL Etests (AB BIODISK, Solna, Sweden) and/or the published clavulanate disk approximation method. A cefepime MIC at ≥4 mg/L was the ESBL screening criterion utilized for *Enterobacter* spp., *Citrobacter* spp. and *Serratia* spp. with confirmation performed using cefepime and clavulanic acid-containing Etests or disks. *K. pneumoniae* ATCC 700603 was used as a QC strain for all ESBL studies.

RESULTS

- Overall, "third-generation" cephalosporins and aztreonam remained active against *E. coli*, *K. oxytoca*, *Serratia* spp. and *Proteus mirabilis* (87.2 to 98.1% susceptible), whereas *K. pneumoniae*, *Enterobacter* spp. and *Citrobacter* spp. showed lower susceptibilities to these agents (69.9 -83.7%).
- The "fourth-generation" agent, cefepime among parenteral cephems, displayed superior activity against Enterobacteriaceae species (89.2 – 98.1% susceptible) primarily due to enhanced stability to the commonly occurring chromosomal and acquired Amp-C ß-lactamases.
- Among all Enterobacteriaceae isolates tested, 11.0% displayed an ESBL phenotype; of those tested by a clavulanic acid inhibition test (1,780 isolates), 76.3% were confirmed as positive, i.e. true ESBLs (Table 2).
- Imipenem and the new glycylcycline, tigecycline, exhibited the broadest spectrum among antimicrobials tested against Enterobacteriaceae, including ESBL-confirmed isolates; imipenem resistance was only detected in *K. pneumoniae* (0.2%; Table 1).
- Screening criteria were most ESBL-specific for *K. pneumoniae* (89.6%), *K. oxytoca* (83.3%), *Citrobacter* spp. (80.0%), *Serratia* spp. (78.1%) and *E. coli* (74.2%), and least specific when testing *Enterobacter* spp. (35.8%), and *P. mirabilis* (57.1%; Table 2).
- The ESBL phenotype rate varied during this 10-year study in *E. coli* (3 [1999] to 8% [2005]) and in *K. pneumoniae* (16 [1999] to 33%[2005]; Figure 1).

Table 1. In vitro activity of select antimicrobial agents tested against seven groups of Enterobacteriaceae isolated in Europe compared with ESBL-confirmed subsets (SENTRY Antimicrobial Surveillance Program, 1997 – 2006).

Organism/antimicrobial agents (no. tested/ESBL-confirmed)	MIC (mg/L): 50%	MIC (mg/L): 90%	MIC (mg/L): Range	% by Category: Overall: Susceptible	Overall: Resistant	ESBL-confirmed: Susceptible[a]	ESBL-confirmed: Resistant[a]
E. coli (15,027/485)							
Ceftazidime	≤2	≤2	≤2->16	97.1	1.9	42.5	37.1
Ceftriaxone	≤0.25	≤0.25	≤0.25->32	96.5	2.8	19.2	72.0
Cefepime	≤0.12	≤0.12	≤0.12->16	98.0	1.6	47.8	41.9
Aztreonam	≤0.12	0.25	≤0.12->16	96.8	2.4	32.6	54.2
Piperacillin/tazobactam	8	8	≤0.5->64	95.2	<0.01	74.0	12.9
Imipenem	≤0.5	≤0.5	≤0.5->8	>99.9[b]	<0.01[b]	100.0	0.0
Ciprofloxacin	≤0.25	>2	≤0.25->2	85.8	14.0	36.9	62.9
Gentamicin	≤2	2	≤2->8	93.7	5.6	56.7	38.6
Tigecycline	0.12	0.25	≤0.03-4	99.9	0.0	100.0	0.0
K. pneumoniae (3,881/652)							
Ceftazidime	≤2	>16	≤2->16	81.0	16.0	26.7	59.2
Ceftriaxone	≤0.25	>32	≤0.25->32	80.2	13.6	19.5	56.6
Cefepime	≤0.12	16	≤0.12->16	89.2	8.0	55.5	33.1
Aztreonam	≤0.12	>16	≤0.12->16	80.0	17.9	19.8	70.2
Piperacillin/tazobactam	2	>64	≤0.5->64	82.6	12.0	51.2	36.3
Imipenem	≤0.25	≤0.5	≤0.5->8	99.5	0.3	99.7	0.2
Ciprofloxacin	≤0.25	>2	≤0.25->2	88.1	10.3	64.4	31.1
Gentamicin	≤2	>8	≤2->8	82.2	16.0	35.5	59.6
Tigecycline	0.5	1	0.06->4	98.8	0.1	99.3	0.0
K. oxytoca (1,121/80)							
Ceftazidime	≤2	>16	≤2->16	95.7	3.5	68.8	27.5
Ceftriaxone	≤0.25	>32	≤0.25->32	91.6	3.9	35.0	28.7
Cefepime	≤0.12	16	≤0.12->16	98.1	1.5	85.0	12.5
Aztreonam	≤0.12	>16	≤0.12->16	87.2	10.5	16.3	71.3
Piperacillin/tazobactam	2	>64	≤0.5->64	84.3	13.2	15.0	77.6
Imipenem	≤0.5	≤0.5	≤0.5->8	100.0	0.0	100.0	0.0
Ciprofloxacin	≤0.25	>2	≤0.25->2	94.3	4.5	70.0	23.9
Gentamicin	≤2	>8	≤2->8	94.7	4.2	70.0	20.0
Tigecycline	0.5	1	0.12-4	99.2	0.0	94.7	0.0
Enterobacter spp. (3,369/68)							
Ceftazidime	≤2	>16	≤2->16	69.9	25.2	17.6	75.0
Ceftriaxone	≤0.25	>32	≤0.25->32	72.1	16.0	0.0	80.9
Cefepime	≤0.5	4	≤0.12->16	96.4	2.2	44.1	29.4
Aztreonam	≤0.12	>16	≤0.12->16	72.6	19.3	8.8	79.4
Piperacillin/tazobactam	4	64	≤0.5->64	73.8	9.9	29.4	27.9
Imipenem	≤0.5	1	≤0.5->8	99.2	0.3	95.6	0.0
Ciprofloxacin	≤0.25	>2	≤0.25->2	84.1	14.2	44.1	51.4
Gentamicin	≤2	8	≤2->8	89.5	8.7	34.3	58.2
Tigecycline	0.5	1	0.06-4	97.3	0.0	91.2	0.0
Citrobacter spp. (683/8)							
Ceftazidime	≤2	>16	≤2->16	82.3	15.8	36.4	54.5
Ceftriaxone	≤0.25	32	≤0.25->32	82.1	6.3	27.3	72.7
Cefepime	≤0.12	1	≤0.12->16	98.1	1.3	36.4	63.6
Aztreonam	≤0.12	16	≤0.12->16	83.7	10.0	9.1	81.8
Piperacillin/tazobactam	2	32	≤0.5->64	86.6	3.8	64.5	27.3
Imipenem	≤0.5	1	≤0.5-8	99.9	0.0	100.0	0.0
Ciprofloxacin	≤0.25	1	≤0.25->2	90.6	8.1	72.7	27.3
Gentamicin	≤2	≤2	≤2->8	92.5	5.8	54.5	36.4
Tigecycline	0.25	0.5	0.12-2	100.0	0.0	100.0	0.0
Serratia spp. (1,188/25)							
Ceftazidime	≤2	≤2	≤2->16	95.3	3.7	59.3	37.0
Ceftriaxone	≤0.25	16	≤0.25->32	88.0	4.6	0.0	70.4
Cefepime	≤0.12	1	≤0.12->16	97.5	1.5	40.7	37.0
Aztreonam	≤0.12	4	≤0.12->16	93.8	4.2	14.8	66.7
Piperacillin/tazobactam	2	32	≤0.5->64	86.2	3.1	74.1	14.8
Imipenem	≤0.5	2	≤0.5->8	99.6	0.2	100.0	0.0
Ciprofloxacin	≤0.25	2	≤0.25->2	89.8	5.5	59.3	22.2
Gentamicin	≤2	8	≤2->8	88.5	2.1	25.9	63.0
Tigecycline	1	1	0.12-4	98.6	0.0	80.0	0.0
Proteus mirabilis (1,590/40)							
Ceftazidime	≤2	≤2	≤2->16	96.1	3.0	80.0	10.0
Ceftriaxone	≤0.25	0.5	≤0.25->32	93.3	3.9	25.0	52.5
Cefepime	≤0.12	0.25	≤0.12->16	96.0	3.3	35.0	57.5
Aztreonam	≤0.12	≤0.12	≤0.12->16	98.1	0.9	85.0	7.5
Piperacillin/tazobactam	≤0.5	1	≤0.5->64	98.1	0.2	97.5	0.0
Imipenem	1	2	≤0.5->8	99.6	0.1	100.0	0.0
Ciprofloxacin	≤0.25	>2	≤0.25->2	81.6	13.0	10.0	80.0
Gentamicin	≤2	>8	≤2->8	84.7	13.1	20.0	72.5
Tigecycline	2	4	0.12->4	56.9	8.2	40.0	26.7

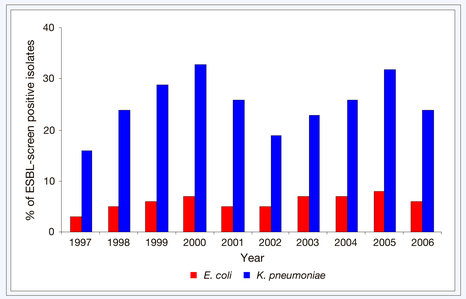
a. Interpretive criteria as recommended by CLSI (M100-S17) for comparison only (ESBL-positive isolates should be reported as resistant to all penicillins, cephalosporins and aztreonam).
b. Only one imipenem-resistant isolate in the entire collection.

Table 2. Prevalence of ESBL producing isolates among Enterobacteriaceae isolated from medical centers in Europe.

Organism (no. tested)	ESBL Screening Criteria Results: No. detected	ESBL Screening Criteria Results: % positive	ESBL Confirmatory Test Results: No. tested	ESBL Confirmatory Test Results: No. (%) positive
E. coli (15,027)	1,013	6.7	654	485 (74.2)
K. pneumoniae (3,881)	1,045	26.9	728	652 (89.6)
K. oxytoca (1,121)	215	19.2	96	80 (83.3)
P. mirabilis (1,590)	172	10.8	70	40 (57.1)
Enterobacter spp. (3,369)	422	12.5[a]	190	68 (35.8)
Citrobacter spp. (683)	31	4.5[a]	10	8 (80.0)
Serratia spp. (1,188)	65	5.5[a]	32	25 (78.1)
Total (26,859)	2,963	11.0	1,780	1,358 (76.3)

a. ESBL phenotype screening criterion of cefepime ≥4 mg/L.

Figure 1. Prevalence of ESBL-phenotype positive *E. coli* and *K. pneumoniae* isolated from European medical centers over a 10 year period of the SENTRY Program.



CONCLUSIONS

- The prevalence of ESBL producing Enterobacteriaceae in Europe has varied in a cyclical manner during this 10-year study, primarily in *K. pneumoniae* and *E. coli* (Figure 1).
- CLSI ESBL-screening criteria best predicted the production of ESBLs among *Klebsiella* spp. (83.3-89.0%) and less so for *P. mirabilis* (57.1%).
- Failure of clinical laboratories to perform ESBL-screen testing may result in the reporting of strains as susceptible to commonly used ß-lactams, with potential for subsequent treatment failures.
- Re-evaluation of cephalosporin breakpoints based upon recent PK/PD and clinical data may help resolve this problem and eliminate the need for ESBL testing and focus efforts toward local infection control activities.
- The continued high prevalence of ESBL enzymes detected among European Enterobacteriaceae is worrisome, warranting continued longitudinal, regional or national monitoring programs (EARSS, SENTRY, etc.).

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