C2-1869

Incidence of ESBL Phenotypes in Citrobacter spp., Enterobacter spp., and S. marcescens Isolates from the SENTRY Antimicrobial Surveillance Program (2001)

A. Mutnick [1,2] M. Beach [1], R.N. Jones [1,3]; [1]The JONES Group/JMI Laboratories, North Liberty, Iowa, USA; [2]University of Iowa, College of Pharmacy, Iowa City, Iowa, and [3]Tufts University School of Medicine, Boston, Massachusetts, USA [www.jmilabs.com]

ICAAC 2002

AMENDED ABSTRACT

Background: The emergence of *Enterobacter*iaceae with ESBLs is a world-wide problem of resistance for "third generation" cephalosporins and monobactams. ESBLs have been reported in numerous *Enterobacter*iaceae including *K. pneumoniae*, *E. coli*, and *P. mirabilis*. This analysis reports the ESBL incidence among *Citrobacter* spp (CB), *Enterobacter* spp (EB), and *Serratia marcescens* (SM) that often also possess high-level third-generation cephalosporin (Amp C) ß-lactamase resistances.
Methods: 1,602 isolates (525 from Europe [EU], 270 Latin America [LA], and 807 North America [NA]) from the 2001 SENTRY Program database were evaluated to identify those isolates with MICs ≥ 8 µg/ml for cefepime (CPM; ESBL phenotypes). CPM was the most sensitive substrate able to detect ESBLs among EBA, CSP, and SM isolates when tested with the ß-lactamase inhibitor, clavulanic acid (CA). Confirmation was defined as a greater than four-fold decrease of the CPM MIC in the presence of 2 µg/ml CA.
Results: A total of 79 isolates (16 EU, 54 LA, and 9 NA [4.9% of the three genus pool]) having MICs ≥ 8 µg/ml of CPM were detected, and were tested with CA for ESBL confirmation. A total of 51 of 79 (65%) isolates confirmed the presence of an ESBL. The incidence of ESBL-producing EBA, CSP, and SM during 2001 was 3.2% of all isolates, regardless of geographic region.
Conclusions: The high volume use of third-generation cephalosporins has resulted in the selection of mutant, resistant populations of bacterial strains possessing chromosomally induced or stably derepressed AmpC ß-lactamases, and this will continue to pose significant problems in the treatment of serious nosocomial infections. In addition, routine evaluation for ESBLs by the clinical laboratory is necessary to proactively direct adequate antimicrobial treatment and infection control. Longitudinal surveillance programs, like SENTRY, encourage the reporting of resistance, and allow for the characterization of resistances with advanced screening microbiologic procedures.

INTRODUCTION

Antimicrobial resistance continues to require longitudinal surveillance systems to assure the ability to communicate the necessary information required by clinical physicians to maximize therapeutic outcomes and guide infection control interventions.

Extended-spectrum-ß-lactamase (ESBL) producing bacteria have been reported in numerous *Enterobacter*iaceae including *Klebsiella* spp., *Escherichia coli*, *Salmonella* spp, *Enterobacter* spp., *Citrobacter* spp., *Serratia marcescens*, and *Proteus mirabilis*. These enzyme-producing strains have demonstrated resistance to extended-spectrum cephalosporins (cefotaxime, ceftazidime) and to monobactams (aztreonam); but to date, have not shown the propensity to confer resistance to cephamycins (cefoxitin, cefotetan) or carbapenems (imipenem, meropenem). However, in those species commonly producing Amp C cephalosporinases (inducible or stably derepressed) such as *Citrobacter* spp., *Enterobacter* spp. and *S. marcescens*, the additional appearance of an ESBL presents unique and problematic therapeutic issues. This report focuses on the occurrence rates of ESBLs in these latter species and a novel, simple method for their detection.

MATERIALS AND METHODS

All strains were tested and interpreted at the SENTRY Program monitoring laboratory using reference broth microdilution methods as described by the National Committee for Clinical Laboratory Standards (M7-A5, 2000; M100-S12, 2002). MIC values were determined using validated, dry-form panels inoculated with the appropriate broth suspension of organisms delivered by an automated inoculator (TREK Diagnostics, Westlake, OH).

A total of 1,602 isolates *Enterobacter* spp., *Citrobacter* spp., and *Serratia marcescens* (525 from Europe, 270 Latin America, and 807 North America) from the year 2001 SENTRY database were evaluated to identify isolates with MICs ≥ 8 µg/ml for cefepime (NCCLS breakpoint for *Enterobacter*iaceae susceptibility at ≤8 µg/ml). This breakpoint was previously determined to have greatest sensitivity with no ESBLs associated with strains having a cefepime MIC at ≤ 4 µg/ml (personal communications, D.J. Biedenbach and R.N. Jones).

Cefepime was the most likely substrate able to detect ESBL enzyme production in *Enterobacter* spp, *Citrobacter* spp., and *Serratia marcescens* isolates when tested with the ß-lactamase inhibitor, clavulanic acid. This fourth-generation cephalosporin retains activity against stably derepressed mutants of these genera (MIC, ≤ 4 µg/ml) while "third-generation" cephalosporins and aztreonam are resistant (MICs, ≥ 8 µg/ml). Phenotypic confirmation of these suspected strains was achieved using Etest ESBL strips (AB BIODISK, Solna, Sweden) containing cefepime ± clavulanic acid. A positive result was identified when a > four-fold decrease was observed in the cefepime MIC when tested in the presence of 2 µg/ml clavulanic acid.

RESULTS

- *Enterobacter* spp. were the most frequent genus showing an ESBL phenotype, and represented 70.9% of screen-positive isolates. *Serratia marcescens* accounted for 27.8% of isolates and *Citrobacter* spp. had only one isolate (1.3%;Table 1).
- The rank order by region for confirmed ESBL-producing *Enterobacter* spp., *Citrobacter* spp., and *Serratia marcescens* was LA (40/270; 14.8%) > EU (7/525; 1.3%) > NA (4/807; 0.5%) (Table 2).
- Isolates from LA demonstrated significantly higher percentages of ESBLs than those from NA or EU, and reflects the higher percentages of ESBLs present within *Klebsiella* spp. (41.1% data not shown), which can serve as a reservoir for dissemination to other Enterobacteriaceae (Table 2).
- *Serratia marcescens* had the highest rate (81.8%) of confirmation for the presence of ESBLs when compared to other species (57.9%), and there was no inter-regional variation (Table 2).
- Cefepime MICs of ≥ 32 µg/ml were most specific (91.4%) for identifying those *Enterobacter*iaceae isolates possessing ESBLs, but the sensitivity was only 44.3%. However, at ≥ 8 µg/ml the screen sensitivity was increased to 100.0%, while the specificity decreased to 64.5%. We recommend the ≥ 8 µg/ml ESBL phenotype screening criteria followed by a confirming test using clavulanate (Table 3).

Table 1. Occurrence of ESBL phenotypes (cefepime MIC, ≥ 8 µg/ml) among *Enterobacter* spp., *Citrobacter* spp., and *S. marcescens* in the SENTRY Antimicrobial Surveillance Program (2001).

Source of isolate (no.)	Organism (no.)	No. (%) confirmed among ESBL phenotypes
Blood stream (49)	*Citrobacter* spp. (1)	1 (100.0)
	Enterobacter spp. (34)	18 (52.9)
	S. marcescens (14)	10 (71.4)
ICU (16)	*Enterobacter* spp. (11)	8 (72.7)
	S. marcescens (5)	5 (100.0)
Respiratory tract (14)	*Enterobacter* spp. (11)	6 (54.5)
	S. marcescens (3)	3 (100.0)
Total (79)	*Citrobacter* spp. (1)	1 (100.0)
	Enterobacter spp. (56)	32 (57.1)
	S. marcescens (22)	18 (81.8)

Table 2. Occurrence and geographic distribution of ESBL phenotypes (cefepime MIC, ≥ 8 µg/ml) in *Enterobacter* spp., *Citrobacter* spp., and *S. marcescens* isolates in three regions of the SENTRY Antimicrobial Surveillance Program (2001).

Region	Organism (no.)	No. ESBL phenotypes	Confirmed no. with ESBL (%)[b]
Europe	*Citrobacter* spp. (69)	1 (1.5)	1 (1.5)
	Enterobacter spp. (349)	11 (3.2)	3 (0.9)
	S. marcescens (107)	4 (3.7)	3 (2.9)
Latin America	*Citrobacter* spp. (22)	0 (-)	0 (-)
	Enterobacter spp. (182)	38 (20.8)	27 (18.8)
	S. marcescens (66)	16 (24.2)	13 (19.7)
North America	*Citrobacter* spp. (104)	0 (-)	0 (-)
	Enterobacter spp. (473)	8 (1.7)	3 (0.6)
	S. marcescens (230)	1 (0.4)	1 (0.4)

a. Using a screening test of a cefepime MIC at ≥ 8 µg/ml.
b. Positive confirming test with cefepime ± clavulanic acid.

Table 3. Relationship between the cefepime MIC and the presence of ESBL-containing isolates.

	Occurrences (%) for:							
	Enterobacter spp.		*Citrobacter* spp.		*S. marcescens*		All strains	
Cefepime MIC (µg/ml)	ESBL +	ESBL -	ESBL +	ESBL -	ESBL +	ESBL -	ESBL +	ESBL -
8	5(26.4)	14(73.6)	0(-)	0(-)	3(100.0)	0(0.0)	8(36.4)	14(63.6)
16	8(53.3)	7(46.7)	1(100.0)	0(0.0)	2(33.3)	4(67.7)	11(50.0)	11(50.0)
≥ 32	20(90.9)	2(9.1)	0(-)	0(-)	12(92.3)	1(7.7)	32(91.4)	3(8.6)
Total	33(58.9)	23(41.1)	1(100.0)	0(100.0)	17(77.3)	5(22.7)	51(64.5)	28(35.5)

CONCLUSIONS

• NA and EU regions demonstrated a very low occurrence rate (0.5 - 1.3%; > 10-fold less) for the studied *Enterobacter*iaceae possessing confirmed ESBL enzymes, as compared to LA (14.8%).

• Unlike the LA region, the majority of ESBL-phenotype isolates among non-*E. coli*, non-*Klebsiella* *Enterobacter*iaceae in NA and EU demonstrated the presence of a resistance mechanism other than an ESBL. Examples include altered outer membrane protein and high levels of stably derepressed AmpC ß-lactamases.

• The ability to maximize therapeutic outcomes in infectious disease patients requires laboratory evaluation for ESBLs in order for clinicians to select the most cost-effective antimicrobial agents. In LA, the high prevalence of ESBLs among Enterobacteriaceae should result in greater use of carbapenems over cefepime, and contrasts with NA and EU regions where the low prevalence of ESBLs in *Enterobacter*s, *Citrobacter*s, and *S marcescens* would favor continued use of cefepime over the carbapenems.

• Longitudinal surveillance programs support the accurate reporting of resistance and the cefepime screening method reported here has a sensitivity of 100.0% and an acceptable level of specificity when used with a confirming test containing clavulanic acid.

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