INFECTIOUS DISEASES

Phenotypic Confirmation of Extended-Spectrum B-Lactamases (ESBL) in Clinical Isolates of *Escherichia coli* and *Klebsiella pneumoniae* at the San Juan Veterans Affairs Medical Center

MARÍA DEL CARMEN RODRÍGUEZ, MT*; DORIS E. VERA, MS, MT**;
CARLOS H. RAMÍREZ-RONDA, MD**; SONIA SAAVEDRA, MD, PhD**

Extended-spectrum Beta (β)-lactamases (ESBLs) have emerged as an important mechanism of resistance to B-lactam antibiotics in gram-negative bacteria (GNB). They are enzymes that hydrolyze older B-lactam antibiotics as well as broad-spectrum cephalosporins and monobactams. ESBL producers have been reported in many bacteria but special attention has been paid to the ones in *E.coli* and *Klebsiella spp.* Detection of the ESBLs by the clinical laboratory is a special challenge. Surveillance to monitor resistance is important to decide when detection of ESBLs must be started. This study determined the prevalence of ESBL producers in the strains *E.coli* and *K.pneumoniae* at the San Juan VA Medical Center, and characterized their phenotypes to evaluate the importance to identify these bacteria as a standard routine procedure in the institution. All *E.coli* and *K.pneumoniae* isolated from Jan 1 to Mar 31, 2003 were evaluated according to National Committee for Clinical Laboratory Standards (NCCLS) screening criteria for suspected ESBL producers. Phenotypic confirmation of the ESBL production was performed using the Etest method. A total of 112/253 (44%) *E.coli* and 72/137 (53%) *K.pneumoniae* were identified as suspected ESBL producers. Etest was performed in 60% of the *E.coli* and 57% of the *K.pneumoniae* suspected to be ESBL producers. The overall ESBL prevalence for *E.coli* was 25% and in *K.pneumoniae* was 26%. Most *E.coli* ESBL-producers were from urine while the *K.pneumoniae* were from sputum. ESBL-producers were isolated from different sources including pleural and synovial fluids, blood, and skin besides urine and sputum. According to susceptibility results, the most reliable antibiotic in predicting a negative ESBL was cefpodoxime (CPD), and in the strains studied, the ESBL producers were consistently resistant to aztreonam (ATM). A large proportion (95%) of ESBL producing *K.pneumoniae* were susceptible to cefepime (CEP). Of the ESBL producing *E.coli*, 24% were susceptible. In the case of *E.coli* ESBL-producers, Cefepime can be considered as a therapeutic option if susceptibilities are available. Automated identification and sensitivity systems are valid alternatives for routine evaluation of B-lactam resistance but when increased resistance is documented in GNB and/or ESBL prevalence is high, ESBL detection should be performed. All confirmed ESBL producers should be reported resistant to all penicillins, cephalosporins, and aztreonam in spite of having susceptible ranges with routine susceptibility tests. Inappropriate antibiotic selection in infections caused by these organisms is associated with treatment failures, poor clinical outcomes, increased mortality and longer hospital stays.

Key words: ESBL, Antimicrobial resistance, Gram-negative bacilli, Susceptibility

One of the most effective survival mechanisms among pathogenic bacteria is antibiotic resistance. There are different mechanisms of antibiotic resistance among bacteria but one of the most important in gram-negative bacteria is the production of β-lactamases, enzymes that destroy β-lactam antibiotics. Beta-lactam antibiotics are among the most important antibacterial armamentarium and are characterized by the presence of a β-lactam ring. Members of this family of antibiotics include the penicillins, cephalosporins, cephamycins, monobactams, carbapenems and the combinations of β-lactam/β-lactamase inhibitors.

There are different types of β-lactamases but extended-spectrum β-lactamases (ESBLs) have emerged as an important mechanism of resistance to ß-lactam antibiotics

From the *Infectious Diseases Research Laboratory, San Juan Veterans Affairs Medical Center, and the ** Department of Clinical Laboratory Sciences, College of Health Related Professions, Medical Sciences Campus, University of Puerto Rico.

Address correspondence to: Sonia Saavedra, MD, PhD, Department of Medicine, Infectious Diseases Section (111), San Juan Veterans Affairs Medical Center, 10 Casia St. San Juan, Puerto Rico 00921-3201. Tel. 787-641-7582 Ext. 10141

in gram-negative bacteria (1). ESBLs are β-lactamases that hydrolyze older β-lactam antibiotics as well as broad-spectrum cephalosporins and monobactams. Attention has been paid mostly to ESBLs in *E.coli* and *Klebsiella spp.* The National Committee for Clinical Laboratory Standards (NCCLS) guidelines refers specifically to *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Escherichia coli* (2, 3). However, ESBL producers have been found in other bacterial genera including other Enterobacteriaceae as *Enterobacter*, *Proteus*, *Citrobacter*, *Morganella*, *Salmonella*, *Providencia*, and *Serratia* species (1, 4, 5, 6, 7). Recovery of antibiotic-resistant strains in some of these other *Enterobacteriaceae* is reported to be increasing, particularly during therapy with a β-lactam agent (1).

Antimicrobial susceptibility testing is one of the most important tasks in the clinical microbiology laboratory (8). It is done daily using standardized methods such as the disk diffusion technique and semiautomated or automated systems based on microdilution techniques (9). However, detection of ESBL producers is a special challenge for clinical microbiology laboratories because although ESBL producer bacteria are able to hydrolyze extended-spectrum penicillins, cephalosporins, and aztreonam, the MICs and disk diffusions tests results for all these antibiotics may be within the accepted susceptible range (10). Special tests such as the double disk test and Etest are required to identify the ESBL producers. NCCLS recommends that ESBL producers be reported as resistant to all penicillins, cephalosporins, and aztreonam even when they are shown to be susceptible to these agents by conventional tests (11, 12). It is important to establish antimicrobial resistance surveillance for the *Enterobacteriaceae* to monitor the trends of resistance among the different members of this family of bacteria. Each institution or laboratory can decide when the special tests to detect ESBL should be started to identify adequately these bacteria and report with accuracy their susceptibility tests results.

Not all laboratories have the ability to recognize ESBLs (13). The ability of the laboratory to detect specific resistance depends on the antibiotics and antibiotic concentrations they include in their susceptibility testing (14). It has been assumed that detection and recognition of this resistance mechanism may be difficult for clinical laboratories when routinely testing organisms specially if they use automated systems (15). On the other hand, identification of ESBL producers by the clinical microbiology laboratories based on the presence of an increased MIC to extended-spectrum cephalosporins and reduction in the MIC in the presence of a β-lactamase inhibitor is expensive and time-consuming. For these reasons, the NCCLS has recommended that these specialized tests be performed only based on the results of screening criteria which include an elevated MIC of one of few cephalosporins (ceftazidime, cefotaxime, cefpodoxime) and/or aztreonam.

Clinically, it is very important that laboratories move to identify ESBL producers since these organisms when present are the cause of concern for clinicians. Since they are reported as susceptible to some penicillins and cephalosporins by the standard microbiological techniques, when they are not identified as ESBLs this leads to an incorrect antibiotic therapy selection and adverse treatment outcomes such as failure to treatment, increased length of stay in hospitals and/or death of patients. These organisms usually remain susceptible only to fourth generation cephalosporins such as cefepime and to carbapenems.

It has also been reported that prevalence of strains expressing the ESBL phenotype may vary with geographical regions. According to the Prevention Project ICARE from the Centers of Disease Control (CDC), the overall rate of ceftazidime (CTZ) resistance in the United States as a whole is 8%, but studies done with *Klebsiella pneumoniae* in different areas have reported higher rates of ESBL production among strains in New York City (44%), Latin America (45%), and Italy (37%) [16]. There is a great geographical diversity even within one country. Results from the SENTRY study also demonstrates the great geographical variations when they report rates of ESBL producer phenotype in Latin America of 45% in *Klebsiella spp.*, 22% in *Proteus spp.*, and 8.5% in *E.coli*, while in Europe were 23%, 11%, and 5.3%, and in United States were 7.6%, 5%, and 3.3% for the same mentioned genera of bacteria (17).

It is important to assess the prevalence and phenotypic characteristics of ESBL producers in the different geographical areas and in a particular hospital. In this study we aimed to determine the prevalence of ESBL producers among the *Eschericha coli* and *Klebsiella pneumoniae* in the San Juan Veterans Affairs Medical Center and characterize their phenotypes to evaluate the importance of establishing the identification of these bacteria as a standard routine test in our institution.

Methods

Bacterial strains. The San Juan VA Medical Center is a 320 bed tertiary care hospital with surgical and medical intensive care units, coronary care unit, hemodyalisis unit, open heart surgery program, spinal cord ward, and serving as acute care site of hospitalization for a large number of nursing home patients. The clinical microbiology laboratory processes over 6,000 clinical cultures annually. Clinical samples (blood, urine, sputum, wound swabs,

catheters, etc.) are processed by conventional methods.

All gram-negative bacteria isolated during the study period (January 1, 2003 to March 31, 2003) at the clinical microbiology laboratory were identified to the species level and their susceptibilities to various antibiotics including cefotaxime (CTX), ceftazidime (CTZ), and aztreonam (ATM) were determined by an automated identification and microdilution system using a panel (Microscan, Dade International Inc., West Sacramento, California). Results were recorded and interpreted according to NCCLS guidelines (11).

Once the clinical microbiology laboratory completed their reports, the Infectious Diseases Research Laboratory collected the organisms for the ESBL evaluation.

Screening tests for suspected ESBL producers. Susceptibilities of all *Klebsiella pneumoniae* and *E. coli* isolated during the study period were reviewed to identify suspected ESBL producers by using the NCCLS criteria (3). All isolates reported by the automated system as intermediate (I) or resistant (R) (MIC > 16 ug/ml) to CTZ, CTX and/or ATM were identified as suspected ESBL producer. All isolates with MIC to CTZ, CTX, and/or ATM > 2 ug/ml but < 8 ug/ml were also identified as a suspected ESBL producer.

API 20E identification. The identification was repeated to all *Klebsiella pneumoniae* and *E.coli* identified as suspected ESBL producer by using an API 20E test (BioMérieux S.A.).

Antimicrobial susceptibility testing. Susceptibilities to CTZ, CTX, and ATM were repeated manually by the Kirby-Bauer disk diffusion test to all suspected ESBL producers identified by the previously described criteria to confirm their susceptibility. The susceptibility of the strains to Cefpodoxime (CPD) and Cefepime (CEP) was also done. These antibiotics were not included in the automated system reports. Results were recorded and interpreted according to NCCLS guidelines (2, 12).

Confirmatory tests. All bacteria identified as suspected ESBL producer were selected to perform the ESBL confirmatory test by the Etest method (AB Biodisk, Solna, Sweden). To detect an ESBL phenotype the Etest was performed in accordance with the guidelines of the manufacturer. The synergistic activity of clavulanic acid (CA) with both CTX and CTZ was confirmed by using two different Etest strips:

CTX, CTX with CA
CTZ, CTZ with CA

Results were interpreted according to NCCLS criteria for *E.coli* and *Klebsiella spp.* isolates (2, 3). Isolates were considered ESBL producers when clavulanate caused a ≥ 3 twofold-concentration decrease (ratio ≥ 8) in the MIC of CTX or CTZ in combination with a CTZ MIC ≥ 1 ug/ml or a CTX MIC ≥ 0.5 ug/ml respectively. Additionally, a strain was considered ESBL positive if a phantom zone or a deformation of the CTX and CTZ zone could be observed, independent of the ratios or MICs. The test was considered non determinable when both MICs were outside the test range of the test device or when the result of one strip was ESBL negative and the result of the other strip was indeterminate. Quality control organisms included *E. coli* ATCC 35218 and *K. pneumoniae* ATCC 700603.

Results

During the study period (January 1, 2003 to March 31, 2003) a total of 953 gram-negative bacteria were isolated at the clinical microbiology laboratory. Of these, 253 (26%) were *E. coli* and 137 (14 %) were *Klebsiella pneumoniae.* When the susceptibilities of all *E. coli* and *Klebsiella pneumoniae* isolated during the study period (390 isolates) were reviewed, a total of 112 *E. coli* and 72 *Klebsiella pneumoniae* were identified as suspected ESBL producers according to NCCLS screening criteria. From the 112 suspected ESBL strains of *E. coli*, 61 (55%) were intermediate or resistant to all three antibiotics (CTZ, CTX, ATM) with MIC's > 16 ug/ml; 7 (6%) were resistant to any two of the three antibiotics but sensitive to the third one; and 44 (39%) were resistant to only one of the three antibiotics and sensitive to the others but with MIC's between 4-8 ug/ml. For the 72 suspected ESBL strains of *Klebsiella pneumoniae*, 50 (69%) were resistant to all three antibiotics; and 22 (31%) were resistant to only one of the three antibiotics and sensitive to the others but with MIC's between 4-8 ug/ml. (Table 1)

Of the 184 strains from both species suspected to be ESBL producers, more than 50%, that is 67/112 (60%) *E. coli* and 41/72 (57%) *Klebsiella pneumoniae*, were available for a confirmation test (Table 1). Of the 108 suspected

Table 1. Bacterial Strains and Screening Tests Results During the Study Period
(January 1, 2003 to March 31, 2003)

	Escherichia coli	*Klebsiella pneumoniae*
Total isolates (953)	253/953 (26%)	137/953 (14%)
Total suspected ESBL	112/253 (44%)	72/137 (53%)
Resistant to all three*	61/112 (55%)	50/72 (69%)
Resistant to any two and sensitive to the other	7/112 (6%)	0/72 (0%)
Resistant to any one but the Sensitive with MIC 4-8 ug ml	44/112 (39%)	22/72 (31%)
Total tested for ESBL	67 /112 (60%)	41 / 72 (57%)

ESBL = Extended-spectrum b-lactamase
*Resistance by MIC to Ceftazidime, Ceftriaxone, and Aztreonam

ESBL producers available for a confirmation test, 49 were from urine samples, 26 were from sputum, 12 from skin, 8 from blood, 7 from catheter tips, and 6 were from other sites as synovial or pleural fluids, abdomen, and others. The distribution of organisms by site in each specie of bacteria is depicted in Table 2.

Table 2. Distribution of suspected ESBL-producing organisms by site of origin

	Escherichia coli	*Klebsiella pneumoniae*	Total
Urine	41	8	49
Sputum	9	17	26
Skin	8	4	12
Blood	3	5	8
Cath tip	3	4	7
Other	3	3	6
TOTAL	67	41	108

Of the *E.coli* suspected to be ESBL producers, 38/67 (57%) resulted positive in the confirmation test, 2/67 (3%) were negative, and 27/67 (40%) were non determinable. Of the *Klebsiella pneumoniae* suspected to be ESBL producers, 20/41 (49%) resulted positive in the confirmation test, 1/41 (2%) was negative, and 20/41 (49%) were non determinable. The overall ESBL prevalence among the *E.coli* was 25% and in *Klebsiella pneumoniae* was 26%. (Table 3)

Table 3. Confirmatory Test Results during the Study Period (January 1, 2003 to March 31, 2003)

	Escherichia coli	*Klebsiella pneumoniae*
Total tested for ESBL	67 /112 (60%)	41 / 72 (57%)
ESBL Positive	38 / 67 (57%)	20 / 41 (49%)
ESBL Negative	2 / 67 (3%)	1 / 41 (2%)
NON-Determinable	27 / 67 (40%)	20 / 41 (49%)
Overall ESBL Prevalence	25%	26%

Susceptibilities were confirmed by the Kirby-Bauer disk diffusion test to CZT, CTX, ATM as well as done to CPD and CEP in all suspected ESBL producers. Result of the susceptibilities is depicted in Tables 4 and 5. All suspected ESBL producer isolates except for 2 strains of *E. coli* and one of *Klebsiella pneumoniae* were resistant to CPD. The

Table 4. Susceptibility for *Escherichia coli* by Automated System (MIC) Disk Diffusion and ESBL Results

Pt #	Site	Susceptibility MIC (UG/ML) / Disk Difussion					ESBL
		CTZ	CTX	ATM	CPD	CEP	
1	B/C	> 16 (R) / I	> 32 (R) / R	> 16 (R) / R	R	R	POS
	U/C	> 16 (R) / R	> 32 (R) / R	> 16 (R) / R	R	R	POS
2	PLEURAL/C	> 16 (R) / I	> 16 (R) / R	> 16 (R) / R	R	I	POS
3	U/C	> 16 (R) / R	> 16 (R) / R	> 16 (R) / R	R	S	POS
4	ULCER/C	> 16 (R) / R	> 32 (R) / R	> 16 (R) / R	R	R	ND
5	U/C	> 16 (R) / R	> 32 (R) / R	> 16 (R) / R	R	R	POS
6	U/C	> 16 (R) / R	> 32 (R) / R	> 16 (R) / R	R	R	POS
7	U/C	> 16 (R) / R	> 32 (R) / R	> 16 (R) / R	R	R	POS
8	U/C	> 16 (R) / R	16 (I) / I	> 16 (R) / R	R	S	POS
9	U/C	> 16 (R) / R	> 32 (R) / R	> 16 (R) / R	R	R	POS
10	S/C	16 (I) / S	> 32 (R) / R	> 16 (R) / R	R	I	ND
11	U/C	> 16 (R) / R	> 32 (R) / R	> 16 (R) / R	R	R	POS
12	U/C	> 16 (R) / R	> 32 (R) / R	> 16 (R) / R	R	R	POS
13	CATH-TIP/C	> 16 (R) / R	> 32 (R) / R	> 16 (R) / R	R	R	ND
	S/C	> 16 (R) / R	> 16 (R) / R	> 16 (R) / R	R	R	ND
	S/C	> 16 (R) / I	> 16 (R) / R	> 16 (R) / R	R	S	POS
14	U/C	> 16 (R) / R	> 16 (R) / R	> 16 (R) / R	R	R	POS
15	WOUND/C	16 (I) / R	> 16 (R) / R	> 16 (R) / R	R	R	ND
16	ULCER/C	> 16 (R) / R	16 (I) / R	> 16 (R) / R	R	R	ND
	S/C	> 16 (R) / R	32 (I) / R	> 16 (R) / R	R	R	ND
17	U/C	> 16 (R) / R	> 32 (R) / R	> 16 (R) / R	R	R	POS
18	SYNOVIAL/C	> 16 (R) / I	> 16 (R) / R	> 16 (R) / R	R	R	POS
19	S/C	> 16 (R) / R	32 (I) / R	> 16 (R) / R	R	R	ND
20	U/C	> 16 (R) / R	32 (I) / R	> 16 (R) / R	R	R	ND
21	ULCER/C	> 16 (R) / R	16 (I) / R	> 16 (R) / R	R	R	ND
22	U/C	> 16 (R) / R	32 (I) / R	> 16 (R) / R	R	R	ND
23	S/C	> 16 (R) / R	> 32 (R) / R	> 16 (R) / R	R	R	ND
24	B/C	> 16 (R) / R	32 (I) / R	> 16 (R) / R	R	S	ND
	U/C	> 16 (R) / R	32 (I) / R	> 16 (R) / R	R	I	ND

Continue

Table 4. Susceptibility for *Escherichia coli* by Automated System (MIC) Disk Diffusion and ESBL Results

Pt #	Site	Susceptibility MIC (UG/ML) / DISK DIFUSSION					ESBL
		CTZ	CTX	ATM	CPD	CEP	
25	U/C	> 16 (R) / R	32 (I) / R	> 16 (R) / R	R	R	ND
	ULCER/C	> 16 (R) / R	16 (I) / R	> 16 (R) / R	R	R	ND
26	CATH/TIP	> 16 (R) / R	> 32 (R) / R	> 16 (R) / R	R	R	POS
27	U/C	> 16 (R) / R	16 (I) / R	> 16 (R) / R	R	R	ND
28	U/C	> 16 (R) / R	> 32 (R) / R	> 16 (R) / R	R	R	POS
29	U/C	> 16 (R) / R	> 16 (R) / R	> 16 (R) / R	R	R	POS
30	U/C	16 (I) / R	> 32 (R) / R	> 16 (R) / R	R	R	POS
31	U/C	> 16 (R) / R	>32 (R) / R	>16 (I) / R	R	R	POS
32	U/C	>16 (R) / R	>32 (R) / R	< 8 (S) / R	R	R	ND
33	U/C	>16 (R) / R	< 4 (S) / S	>16 (R) / R	R	S	POS
34	U/C	>16 (R) / R	< 4 (S) / S	16 (I) / R	R	S	POS
35	ULCER/C	> 16 (R) /R	< 4 (S) / S	>16 (R) / R	R	S	POS
36	U/C	> 16 (R) /R	< 4 (S) / S	>16 (R) / R	R	S	POS
37	CATH-TIP/C	> 16 (R) /R	< 4 (S) / R	16 (I) / R	R	I	POS
38	S/C	>16 (R) / I	8 (S) / R	>16 (R) / R	R	R	ND
39	U/C	16 (I) / I	8 (S) / R	>16 (R) / R	R	R	POS
40	U/C	16 (I) / R	8 (S) / I	16 (I) / R	R	S	POS
41	ULCER/C	>16 (R) / R	8 (S) / R	>16 (R) / R	R	R	POS
42	S/C	8 (S) / R	16 (I) / R	>16 (R) / R	R	R	ND
43	U/C	8 (S) / I	8 (S) / R	>16 (R) / R	R	R	POS
44	U/C	8 (S) / R	16 (I) / R	>16 (R) / R	R	R	ND
S/C	16 (I) / R	8 (S) / R	>16 (R) / R	R	R	POS	
45	U/C	8 (S) / R	8 (S) / R	>16 (R) / R	R	R	ND
46	U/C	>16 (R) / R	8 (S) / R	>16 (R) / R	R	R	POS
47	U/C	8 (S) / R	< 4 (S) / I	>16 (R) / R	R	R	ND
48	U/C	8 (S) / I	> 32 (R) /R	>16 (R) / R	R	R	POS
49	U/C	>16 (R) / I	8 (S) / I	>16 (R) / R	R	S	POS
50	U/C	8 (S) / S	8 (S) / S	>16 (R) / S	S	S	NEG
51	U/C	>16 (R) / R	8 (S) / R	>16 (R) / R	R	R	POS
52	U/C	>16 (R) / R	8 (S) / R	>16 (R) / R	R	I	POS
53	SYNOVIAL/C	>16 (R) / R	8 (S) / R	>16 (R) / R	R	R	POS
B/C	16 (I) / R	8 (S) / R	>16 (R) / R	R	R	POS	
54	U/C	>16 (R) / R	8 (S) / R	>16 (R) / R	R	R	POS
55	U/C	8 (S) / S	32 (I) / S	>16 (R) / S	S	S	NEG
56	U/C	8 (S) / I	8 (S) / R	>16 (R) / R	R	R	ND
57	ULCER/C	8 (S) / R	< 4 (S) / I	>16 (R) / R	R	S	ND
58	U/C	16 (I) / R	8 (S) / R	>16 (R) / R	R	I	ND
59	U/C	>16 (R) / R	8 (S) / R	>16 (R) / R	R	I	ND

Table 5. Susceptibility for *Klebsiella pneumoniae* by automated system (MIC) and Disk Diffusion, and ESBL results

PT #	SITE	Susceptibility MIC (UG/ML) / Disk Difussion					ESBL
		CTZ	CTX	ATM	CPD	CFP	
1	B/C	> 16 (R) / R	> 32 (R) /R	> 16 (R) / R	R	R	POS
2	B/C	> 16 (R) / R	32 (I) / R	> 16 (R) / R	R	I	ND
	ABDOMEN/C	> 16 (R) / R	16 (I) / R	> 16 (R) / R	R	R	ND
3	Cath tip/C	> 16 (R) / R	> 32 (R) /R	> 16 (R) / R	R	R	ND
4	S/C	> 16 (R) / R	16 (I) / I	> 16 (R) / R	R	S	POS
5	S/C	> 16 (R) / R	> 32 (R) /R	> 16 (R) / R	R	R	ND
6	S/C	> 16 (R) / R	16 (I) / R	> 16 (R) / R	R	R	ND
7	ULCER/C	> 16 (R) / R	16 (I) / I	> 16 (R) / R	R	S	POS
8	U/C	> 16 (R) / R	32 (I) /R	> 16 (R) / R	R	I	ND
	B/C	> 16 (R) / R	32 (I) /R	> 16 (R) / R	R	R	ND

Continue

Table 5. Susceptibility for *Klebsiella pneumoniae* by automated system (MIC) and Disk Diffusion, and ESBL results

PT #	SITE	Susceptibility MIC (UG/ML) / Disk Difussion					ESBL
		CTZ	CTX	ATM	CPD	CFP	
9	S/C	> 16 (R) / R	32 (I) /R	> 16 (R) / R	R	S	POS
10	B/C	> 16 (R) / R	16 (I) /R	> 16 (R) / R	R	S	ND
11	S/C	> 16 (R) / R	16 (I) /R	> 16 (R) / R	R	R	ND
12	U/C	> 16 (R) / R	32 (I) /R	> 16 (R) / R	R	R	ND
13	S/C	> 16 (R) / R	32 (I) /R	> 16 (R) / R	R	R	ND
14	SYNOVIAL/C	> 16 (R) / R	> 16 (R) / R	> 16 (R) / R	R	R	ND
15	S/C	> 16 (R) / R	32 (I) /R	> 16 (R) / R	R	R	ND
16	U/C	> 16 (R) / R	16 (I) /R	> 16 (R) / R	R	S	POS
	S/C	> 16 (R) / R	16 (I) /R	> 16 (R) / R	R	S	POS
17	S/C	> 16 (R) / R	32 (I) /R	> 16 (R) / R	R	S	POS
18	S/C	> 16 (R) / R	16 (I) / I	> 16 (R) / R	R	S	POS
19	S/C	> 16 (R) / R	32 (I) /R	> 16 (R) / R	R	R	ND
	Cath/C	> 16 (R) / R	32 (I) /R	> 16 (R) / R	R	R	ND
20	S/C	> 16 (R) / R	32 (I) /R	> 16 (R) / R	R	R	ND
21	U/C	> 16 (R) / R	32 (I) /R	> 16 (R) / R	R	R	ND
22	S/C	> 16 (R) / R	32 (I) /R	> 16 (R) / R	R	S	POS
23	S/C	> 16 (R) / R	16 (I) /R	> 16 (R) / R	R	S	POS
24	U/C	> 16 (R) / R	32 (I) /R	> 16 (R) / R	R	S	POS
25	Cath/C	> 16 (R) / R	32 (I) /R	> 16 (R) / R	R	R	ND
26	S/C	> 16 (R) / R	8 (S) / I	> 16 (R) / R	R	S	POS
27	S/C	> 16 (R) / I	8 (S) / I	> 16 (R) / R	R	S	POS
28	S/C	> 16 (R) / R	8 (S) / I	> 16 (R) / R	R	S	POS
29	B/C	8 (S) / I	< 4 (S) / I	> 16 (R) / R	R	I	ND
30	BRUSH/C	4 (S) / S	8 (S) / S	> 16 (R) / S	S	S	NEG
31	U/C	> 16 (R) / R	8 (S) / I	> 16 (R) / R	R	S	POS
32	U/C	4 (S) / S	8 (S) / I	16 (I) / I	R	S	POS
33	SKIN/C	> 16 (R) / R	8 (S) / R	> 16 (R) / R	R	S	POS
34	SKIN/C	> 16 (R) / R	8 (S) / R	> 16 (R) / R	R	R	ND
35	U/C	> 16 (R) / R	8 (S) / R	> 16 (R) / R	R	S	POS
37	CATH TIP/C	> 16 (R) / R	8 (S) / I	> 16 (R) / R	R	S	POS
38	ULCER/C	> 16 (R) / R	8 (S) / I	> 16 (R) / R	R	S	POS

susceptibilities for CEP and CPD correlated to ESBL results are depicted in Table 6. In the thirty-eight ESBL confirmed *E. coli* strains, 29 (76%) resulted resistant to CEP, and 9 (24%) resulted susceptible. The two strains that were negative for ESBL were susceptible to CEP. For the non-determinable strains 25/27 (93%) were resistant to CEP and 2/27 (7%) were susceptible. For the confirmed ESBL producers *Klebsiella pneumoniae* strains, only 1/20 (5%) resulted resistant to CEP, and 19/20 (95%) were susceptible. The strain that was negative for ESBL was susceptible to CEP. In the non-determinable strains, 95% (19/20) were resistant to CEP and 5% (1/20) were susceptible. Of all the 47 strains that resulted non-determinable for ESBL, 94% (44/47) were resistant to CEP and only 6% (3/47) were susceptible. For CPD, all isolates were resistant and the only three isolates susceptible were the three strains that were negative for ESBL.

Table 6. Susceptibility to Cefepime (CEP) and Cefpodoxime (CPD) according to ESBL results

	Escherichia coli			*Klebsiella pneumoniae*		
ESBL result	**Positive**	**Negative**	**N D**	**Positive**	**Negative**	**N D**
Resistant to CEP	29/38 (76%)	0/2 (0%)	25/27 (93%)	1/20 (5%)	0/1 (0%)	19/20 (95%)
Sensitive to CEP	9/38 (24%)	2/2 (100%)	2/27 (7%)	19/20 (95%)	1/1 (100%)	1/20 (5%)
Resistant to CPD	38/38 (100%)	0/2 (0%)	27/27 (100%)	20/20 (100%)	0/1 (0%)	20/20 (100%)
Sensitive to CPD	0/30 (0%)	2/2 (100%)	0/27 (0%)	0/20 (0%)	1/1 (100%)	0/20 (0%)

N D = Non Determinable

Discussion

The study findings revealed an overall prevalence of ESBL producers among the *E._coli* and *Klebsiella pneumoniae* strains of 25% and 26% respectively. In this study of the 67 *E. coli* strains tested for ESBL, 57% (38/67)

actually contained an ESBL. In the 41 *Klebsiella pneumoniae* strains tested, 49% (20/41) contained an ESBL.

Of the ESBL producers *E. coli*, 71% (27/38) were from urine. In *Klebsiella pneumoniae*, 50% (10/20) were from sputum. This was not surprising since the most common organisms isolated in urine is *E. coli* and *Klebsiella pneumoniae* is an organism commonly isolated from sputum.

The present NCCLS recommendations are to screen for ESBL production in *E. coli* and *Klebsiella spp.* using criterion of an MIC of $\geq$ 2 ug/ml for one of the following four agents: CTZ, ATM, CTX, or ceftriaxone, and $\geq$ 8 ug/ml for CPD. In our study we did not examined susceptibility to ceftriaxone, but results of susceptibility to the other four antibiotics demonstrated that the highest susceptibility variations were with CTX followed by CTZ. All suspected ESBL producers were consistently resistant to ATM. The most useful antibiotic in predicting a negative ESBL was CPD, all ESBL producers were resistant and all non-ESBL producers were sensitive. These CPD susceptible isolates were also susceptible to all antibiotics tested by the Kirby-Bauer method, but not in the test by the automated system. They were initially included in the suspected ESBL producers according to the MIC criteria supporting the importance of performing ESBL confirmatory tests in all suspected ESBL producers. CPD results were not surprising since CPD is a good substrate for all ESBLs and can be used as a sole agent for screening for ESBL to perform confirmatory tests only in all resistant isolates.

The correlation between the susceptibilities reported by the automated system and the ones reported by the Kirby-Bauer disk diffusion method was excellent except for the strains reported susceptible with MIC's of 8 ug/ml that in most of the cases resulted resistant by the Kirby-Bauer method. This was observed most frequently with CTZ and CTX. It is clear that if organisms are confirmed to be ESBL producers, they should be reported as resistant to all, penicillins, cephalosporins, and aztreonam. However, our observations suggest that even though an organism is not confirmed to produce an ESBL, in organisms with susceptibilities $\geq$ 8 ug/ml to these antibiotics the antibiotic should be used cautiously clinically and if possible not used.

The results of susceptibility to CEP were interesting. Most of the ESBL producing *Klebsiella pneumoniae* (95%) were susceptible to CEP, and only one strain positive for ESBL resulted resistant to CEP. However this is not the case for strains of *E. coli* that produce ESBL in which 76% resulted resistant to CEP and 24% were susceptible. The use of fourth generation cephalosporins in the treatment of patient with ESBL producing bacteria is only recommended if susceptibility results show that the organism is susceptible to them. According to our results it appears that there is no problem in using this antibiotic for ESBL producing *Klebsiella pneumoniae*, but susceptibility results of CEP for ESBL producing *E.coli* are necessary before deciding to treat a patient with infection due to this organism due to the high percent of resistance among this group of organisms. For the organisms that resulted non-determinable in the ESBL confirmatory test (47/108 or 44% of all organisms tested for ESBL), resistance to CEP was present in 93% of the strains or more in both *E. coli* and *Klebsiella pneumoniae*. A non-determinable result may suggest additional mechanisms of resistance that can mask clavulanic acid inhibition such as the presence of an IRT (inhibitor-resistant TEM) ESBL, TEM and SHV b-lactamases with reduced affinities for b-lactamases inhibitors, Amp C enzymes, porin changes, or MIC values outside the test device range.

The large number of non-determinable results obtained suggests that ESBL phenotypes are increasingly complex due to production of multiple enzymes and/or other resistance mechanisms. It has been reported that detection of ESBL-producing strains is more difficult in those strains for which ESBL are atypical or when superimposed with other resistance mechanisms (18). Confirmation tests based on testing with both ceftazidime and cefotaxime with and without clavulanic acid may not be sufficient for ESBL detection. Some new Etest ESBL strips based on other antibiotics such as cefepime with clavulanic acid are being investigated as a valuable alternative to current methods for detection of ESBLs (19). There are preliminary studies that suggests that cefepime based tests are more sensitive than ceftazidime and cefotaxime for the detection of ESBL in species other than *E. coli* including *Klebsiella pneumoniae*, *P. mirabilis*, *E. cloacae*, *E. aerogenes*, and *S. marcesens* with non-determinable ESBL results.

Conclusions

β-lactam resistance among clinical isolates is a growing problem. ESBLs in gram-negative bacteria are causing serious resistance problems worldwide (1). Identification of ESBLs and other emerging β-lactamases will soon be imperative for clinical microbiology laboratories. There is a need for guidelines for ESBL testing of species other than *E. coli* and *Klebsiella pneumoniae*. There is also need to develop more laboratory tests to confirm ESBL production and discriminate between the different types of enzymes conferring resistance to these antibiotics.

Automated systems are valid alternatives for routine

evaluation of β-lactam resistance in clinical microbiology laboratories. However, surveillance most be done to identify early increases in resistance or progressive increase in MICs among gram-negative bacteria. It is important to evaluate locally the tests chosen to adapt the diagnostic tests for ESBL detection to a particular geographic area, particular hospital, and particular genera of bacteria.

Even though the known limitations of the phenotypic methods, they are currently the simplest and most cost-effective strategies for detection of ESBLs among gram-negative bacteria.

Infections with ESBL-producers are associated with longer hospital stays, greater hospital costs, treatment failures, suboptimal clinical outcomes, and increased mortality. ESBL producing isolates should be reported as resistant to all penicillins, cephalosporins, and aztreonam in spite of having MICs in susceptible range to this antibiotics. It still unclear if this is true also for the β-lactam/β-lactamase inhibitor combinations.

Resumen

Las β-lactamasas de espectro extendido han surgido como un mecanismo importante de resistencia a los antibióticos β-lactámicos entre las bacterias gram-negativas. Son enzimas que hidrolizan los antibióticos β-lactámicos originales así como las cefalosporinas incluyendo las de espectro extendido y los monobactámicos. Organismos productores de estas enzimas se han reportado en diferentes especies de bacterias pero han tenido una atención especial las cepas de *E.coli* y especies de *Klebsiella*. La detección de estas enzimas ha sido un reto para los laboratorios clínicos. La vigilancia para seguir la resistencia en una institución en particular es importante para decidir en que momento se debe comenzar la detección de los organismos productores de β-lactamasas de espectro extendido. En este estudio se determinó la prevalencia de productores de β-lactamasas de espectro extendido en las cepas de *E.coli* y *Klebsiella pneumoniae* del Hospital de Veteranos de San Juan y se caracterizaron sus fenotipos para evaluar la importancia de identificar estas bacterias como rutina en la institución. Todas la cepas de *E.coli* y *Klebsiella pneumoniae* aisladas desde enero 1 hasta marzo 31 del 2003 fueron evaluadas de acuerdo a los criterios de cernimiento para organismos sospechosos de producción de estas enzimas establecidos por el Comité Nacional de estandards para Laboratorios clínicos. El método de Etest se utilizó en todas las cepas sospechosas de producir dichas enzimas para la confirmación fenotítipa de la producción de las mismas. Un total de 112/253 (44%) de las *E.coli* y 72/137 (53%) de las *K.pneumoniae* fueron identificadas como sospechosas de producir β-lactamasas de espectro extendido. La prueba de Etest se le aplico al 60% de las *E.coli* y al 57% de las *K. pneumoniae* sospechosas de producir la enzima. La prevalencia de producción de β-lactamasas de espectro extendido en *E.coli* fue de 25% y en *K.pneumoniae* de 26%. La mayoría de las cepas de *E.coli* productoras de estas enzimas eran de muestras de orina mientras que las de *K.pneumoniae* eran de esputo, sin embargo en ambos casos hubo organismos productores de enzimas aislados de otras fuentes como fueron líquidos pleurales o sinoviales, sangre y piel además de orina y esputo. De acuerdo a los resultados de susceptibilidades, el antibiótico que resultó ser el más predictivo de ausencia de producción de β-lactamasas de espectro extendido fue cefpodoxima. En las cepas aisladas en el Hospital de Veteranos, la resistencia a aztreonam fue consistente en todas las cepas productoras de la enzima. Mientras el 95% de las *K.pneumoniae* productoras de la enzima resultaron susceptibles a cefepime, solo el 24% de las cepas de *E.coli* productoras de la enzima fueron susceptibles a este antibiótico. El uso clínico de cefepime en cepas de *E.coli* productoras de esta enzima debe estar limitado a la disponibilidad de susceptibilidades que indiquen que la cepa es susceptible. Los sistemas automatizados para la identificación y susceptibilidad a antibióticos son alternatives aceptables para la evaluación rutinaria de resistencia a β-lactámicos pero cuando esta resistencia aumenta entre los organismos gram-negativos o la prevalencia de cepas productoras de β-lactamasas de espectro extendido es alta, se deben hacer pruebas para confirmar la producción de dichas enzimas. Todas las bacterias a las cuales se les confirme la producción de dichas enzimas deben ser reportadas como resistentes a todas las penicilinas, cefalosporinas y aztreonam irrespectivo de los resultados obtenidos en las pruebas de rutina de susceptibilidades a estos antibióticos. La selección inapropiada de antibióticos para tratar infecciones causadas por estos organismos esta asociada con fallas a tratamiento, pobres resultados clínicos, aumento en mortalidad y estadías prolongadas en los hospitales.

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