Antibacterial Susceptibility of Extended-Spectrum Beta-Lactamase-Producing *Klebsiella pneumoniae* and *Escherichia coli*

Jihad Bishara MD[1], Gilat Livne MD[2], Shai Ashkenazi MD[2], Itzhak Levy MD[2], Silvio Pitlik MD[1], Orit Ofir MSc[3], Bruria Lev MSc[3] and Zmira Samra PhD[3]

[1]Infectious Diseases Unit and [3]Department of Clinical Microbiology, Rabin Medical Center (Beilinson Campus), Petah Tiqva, Israel
[2]Infectious Diseases Unit, Schneider Children's Medical Center of Israel, Petah Tiqva, Israel
Affiliated to Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

Key words: antibiotics, *Escherichia coli*, extended spectrum β-lactamase, *Klebsiella pneumoniae*, resistance

Abstract

Background: The prevalence of extended-spectrum β-lactamase-producing organisms and their antimicrobial resistance patterns may vary between geographic areas.

Objectives: To evaluate the prevalence and susceptibility of ESBL-producing organisms among *Klebsiella pneumoniae* and *Escherichia coli* isolated from adult and pediatric patients in two Israeli hospitals.

Methods: ESBL production was tested according to recommendations of the Clinical and Laboratory Standards Institute, using ceftazidime (30 μg) and a combination of ceftazidime/clavulanate (30/10 μg) disks with a ⩾5 mm difference indicating positivity. Antibiotic susceptibilities were determined by the disk diffusion method according to CLSI standards. Minimal inhibitory concentrations were determined by the E-test.

Results: The prevalence of ESBL-producing organisms was significantly higher among *K. pneumoniae* than *E. coli* isolates – 32% (241/765) vs. 10% (57/547) respectively ($P < 0.001$), and more frequently isolated from adults than children (odds ratio 2.27 for *K. pneumoniae* and 12.94 for *E. coli*). Resistance rates for amoxicillin/clavulanate, piperacillin-tazobactam, amikacin, and ciprofloxacin among the ESBL-producing *K. pneumoniae* and *E. coli* isolates were 95%, 82%, 49% and 77% for *K. pneumoniae*, and 77%, 35%, 25% and 100% for *E. coli*. Two (0.8%) ESBL-producing and 4 (0.7%) ESBL-negative *K. pneumoniae* isolates showed intermediate susceptibility (MIC 6 μg/ml) to meropenem. All isolates were sensitive to ertapenem and colistin.

Conclusion: ESBL production among *K. pneumoniae* and *E. coli* is more prevalent in the adult population than the pediatric population and is associated with multidrug resistance.

IMAJ 2005;7:298–301

For Editorial see page 336

During the past 60 years, bacteria have demonstrated a remarkable ability to resist almost every antibiotic that has been developed. Four major mechanisms of resistance have been described: target site alterations (such as changes to the penicillin-binding protein), inactivation of antimicrobials (as by penicillinases or the new carbapenemases), alterations in cell wall permeability, and pumping the antimicrobial out of the cell by efflux mechanisms before it can reach its target site.

Antimicrobial resistance of Enterobacteriaceae species to β-lactam antibiotics usually results from the production of enzymes that hydrolyze the β-lactam ring of these antibiotics [1]. To overcome this problem, extended spectrum cephalosporins were introduced in the mid-1980s. Subsequently, a new group of enzymes, the ESBL, emerged in numerous Enterobacteriaceae in many hospitals worldwide [2,3]. These enzymes confer resistance to extended-spectrum cephalosporins and related oximino-β-lactams (ceftazidime, ceftriaxone, cefoxitin, aztreonam), remaining generally susceptible to carbapenems, cephamycins, and β-lactamase inhibitors such as clavulanic acid [3]. The vast majority of ESBLs are derivatives of TEM-1 (the common plasmid-mediated β-lactamase of organisms such as *E. coli*) or SHV-1 (the common chromosomally mediated β-lactamase of *K. pneumoniae*) [4].

Several risk factors for acquiring infections by ESBL-producing organisms in both hospital and community settings have been reported in the literature. These infections are more common after prolonged hospitalization (especially in intensive care units), residence in a nursing home, previous broad-spectrum antibiotic therapy (mainly cephalosporins), old age, co-morbidities, and others [3,5,6]. If treated with inappropriate antibiotics, the mortality rate of patients with serious infections due to ESBL-producing organisms is significantly higher than in patients treated with appropriate antibiotics (ranging from 42% to 100%) [4]. Clinical outcome is poor when third-generation cephalosporins are used to treat infections with ESBL-producing organisms, even in the presence of apparent "susceptibility" [6]. Carbapenems have been shown to be associated with the lowest mortality rate and are regarded as the drug of choice for serious infections with ESBL-producing organisms [7].

The prevalence of extended spectrum β-lactamase-producing bacteria in many hospitals has increased worldwide during the past

ESBL = extended spectrum β-lactamase
CLSI = Clinical and Laboratory Standards Institute (formerly the NCCLS)
MIC = minimal inhibitory concentration

Table 1. Distribution and prevalence of ESBL-producing *K. pneumoniae* and *E. coli* isolates in children and adult patients

	All		Adults		Children				
Microorganism	**Total**	**ESBL+ (%)**	**Total**	**ESBL+ (%)**	**Total**	**ESBL+ (%)**	***P***	**OR**	**95%CI**
K. pneumoniae	765	241 (32)	536	195 (36)	229	46 (20)	0.000	2.27	1.57–3.29
E. coli	547	57 (10)	454	56 (12)	93	1 (1)	0.002	12.94	1.77–94.73

ESBL+ = extended spectrum β-lactamase-positive.

few years [2]. Since the production of ESBLs usually coincides with a wide-range resistance pattern to antimicrobials, treatment to eradicate these microorganisms has become more challenging. *K. pneumoniae* is by far the most common species in which ESBL has been recognized, accounting for 75% of ESBL-producing isolates. Currently, plasmid-mediated ESBL production has been detected in 14–16% of *K. pneumoniae* isolates in Europe. *E. coli* and rarely *Proteus mirabilis* are among the ESBL-producing *Enterobacteriaceae* species [2].

Table 2. Antimicrobial resistance of ESBL-positive vs. ESBL-negative strains among *K. pneumoniae* and *E. coli* isolates (%)

	K. pneumoniae			*E. coli*		
	ESBL+ n=241	**ESBL-n=524**	***P***	**ESBL+ n=57**	**ESBL-n=490**	***P***
Amoxicillin/clavulanate	229 (95)	190 (36)	<0.001	44 (77)	136 (28)	<0.01
Piperacillin-tazobactam	197 (82)	178 (34)	<0.001	20 (35)	27 (5.5)	<0.01
Amikacin	117 (49)	37 (7)	<0.001	14 (25)	27 (5.5)	<0.01
Ciprofloxacin	186 (77)	87 (16)	<0.001	57 (100)	99 (20)	<0.01
Imipenem	1 (0.4)	4 (0.7)	NS	0 (0)	0 (0)	
Meropenem	2 (0.8)	4 (0.7)	NS	0 (0)	0 (0)	
Ertapenem	0 (0)	0 (0)		0 (0)	0(0)	
Colistin	0 (0)	0 (0)		0 (0)	0 (0)	

ESBL+ = extended spectrum β-lactamase-positive, ESBL- = extended spectrum β-lactamase-negative, NS = not significant.

There are geographic variations in the prevalence of ESBLs among the *Enterobacteriaceae* family between countries, regions, and hospitals within the same country. Published data on ESBL-producing organisms in Israel are scarce [3,5,8–11]. These studies focused on risk factors and prevalence of community-acquired ESBL-producing organisms [5,9], and on the impact of ESBL-producing organisms on mortality from nosocomial bacteremia [3]. Two reports on susceptibility patterns of ESBL-producing bacteria in northern Israel [11] and the Tel Aviv area [10] were recently published.

The objectives of this study were to evaluate the prevalence of ESBL-producing organisms among *K. pneumoniae* and *E. coli* isolated from hospitalized adult and pediatric patients in two university hospitals in central Israel, and to assess the *in vitro* antimicrobial susceptibility of these organisms, in order to create a solid database for the appropriate use of antibiotics when such infections are suspected in tertiary centers in Israel.

Materials and Methods

The study was conducted at the Rabin Medical Center (Beilinson Campus, 950 beds) and the neighboring Schneider Children's Medical Center of Israel (250 beds) – two university-affiliated tertiary care facilities in central Israel. During a 6 month period, all consecutive isolates of *Klebsiella* sp. and *E. coli* recovered from sterile (blood and urine) and non-sterile sites (wounds and sputum) were studied prospectively. Blood cultures were processed using the Bactec 9240 blood culture system (Beckton Dickinson, USA). Standard methods were used to culture clinical specimens. Isolates were identified using routine bacteriologic procedures. Extended spectrum β-lactamase production was tested according to recommendations of the Clinical and Laboratory Standards Institute, using ceftazidime (30 μg) and a combination of ceftazidime/clavulanate (30/10 μg) disks (oxoid), with a $\geqslant$5 mm difference indicating positivity. Antibiotic susceptibility was determined by the disk diffusion method according to CLSI standards [12]. MIC was determined by the E-test (AB Biodisk, Solna, Sweden).

Statistical analysis

The significance of the differences in ESBL production and antibiotic resistance was determined by the chi-square test with Yates correction for continuity, using the Compare 2 software (J.H. Abramson, 2000-2001).

Results

Altogether, 765 *K. pneumoniae* and 547 *E. coli* isolates were recovered. Forty-seven percent (363/765) of *K. pneumoniae* and 65% (355/547) of *E. coli* isolates were recovered from sterile sites. The prevalence of ESBL-producing organisms was significantly higher among the *K. pneumoniae* isolates than the *E. coli* – 32% (241/765) vs. 10% (57/547) respectively ($P < 0.001$, odds ratio 3.95, 95% confidence interval 2.89–5.41). ESBL-producing *K. pneumoniae* and *E. coli* were isolated most commonly from wounds (42% and 14%, respectively). Among bloodstream isolates, ESBL-producing *K. pneumoniae* and *E. coli* accounted for 27% and 10% of isolates, respectively. The rate of ESBL-producing organisms among urinary isolates of *E. coli* was low, accounting for only 6% compared to 35% for *K. pneumoniae*. The rate of ESBL-producing organisms among sputum isolates of *K. pneumoniae* was 18%. The overall distribution and prevalence of ESBL-production among both organisms isolated from children and adult patients are shown in Table 1.

The percentage of ESBL-producing organisms among *K. pneumoniae* and *E. coli* isolates was significantly higher in adults

CI = confidence interval

than in pediatric patients with an odds ratio of 2.27 (95% CI 1.57–3.29, $P < 0.000$) for *K. pneumoniae* and 12.94 (95%CI 1.77–94.73, $P = 0.002$) for *E. coli*. In children, the rate of ESBL-producing *E. coli* was very rare (1%) [Table 1].

The frequency of resistance to antimicrobials tested for ESBL-producing and ESBL-non-producing *K. pneumoniae* and *E. coli* isolates is presented in Table 2. The prevalence of multidrug-resistant strains among ESBL-producing *K. pneumoniae* is striking and significantly higher than the prevalence found among the ESBL-producing *E. coli* strains, except for ciprofloxacin [Table 2]. A very high resistance (>80%) to piperacillin-tazobactam was found among ESBL-producing *K. pneumoniae*.

A worrisome finding was the emergence of carbapenem non-susceptible strains among *K. pneumoniae* [Table 2]. Klebsiella strains that were resistant to meropenem by the disk diffusion method had an MIC to meropenem of 6 mg/L. According to the CLSI, such strains should be categorized as having intermediate susceptibility. All ESBL-producing isolates among *K. pneumoniae* and *E. coli* were sensitive to ertapenem as well as to colistin [Table 2].

Discussion

To the best of our knowledge, this is the first study comparing the prevalence of ESBL production among *K. pneumoniae* and *E. coli* isolated from sterile and non-sterile sites, in adult and children patients, from two tertiary hospitals located on the same campus and sharing the same microbiology laboratory. In this study, similar to several previous reports [3,5,8,9,13,14], ESBL production was significantly higher among *K. pneumoniae* than *E. coli* isolates. The highest prevalence of ESBL was among wound isolates (42% for *K. pneumoniae* and 14% for *E. coli*).

These findings are much higher than those reported in the SENTRY study from North America [15]. The prevalence of ESBL-producing organisms among bloodstream isolates in our institutions was high, but comparable to those reported from other institutions in Israel [8] and other countries [16] and higher than others [17]. It should be emphasized that the real prevalence of ESBL-producing organisms among the tested *K. pneumoniae* and *E. coli* isolates could be underestimated due to the reduced sensitivity of the applied method (one substrate, ceftazidime) for testing ESBL production in our study. Interestingly, the prevalence of ESBL production for both organisms was significantly higher in adults than in children. The rate of ESBL-producing *E. coli* in pediatric patients was very low.

In comparison with previous reports on children, most studies were conducted on a selective neonatal population [18], on patients in pediatric intensive care [19] and in transplantation units [20]. According to these studies, very high rates of ESBL production were found among bloodstream Klebsiella sp. (87%) and *E. coli* (64%) isolates [20].

The most striking finding of the present study was the very high prevalence of resistant strains among ESBL-producing *K. pneumoniae* to piperacillin-tazobactam, and among ESBL-producing *E. coli* to ciprofloxacin. A study group from southern Israel reported zero resistance to piperacillin-tazobactam among their community-acquired ESBL-producing Enterobacteriaceae blood isolates [9], and only 35% resistance among nosocomial ESBL-producing Enterobacteriaceae blood isolates [3]. Colodner et al. [11], in their recently published study on the susceptibility patterns of ESBL-producing Enterobactereaceae in northern Israel, reported a 7% resistance to piperacillin-tazobactam. In these studies, antimicrobial susceptibilities of ESBL-producing *K. pneumoniae* and *E. coli* were not specified. On the other hand, a group from Tel Aviv found that 60% of their ESBL-producing Klebsiella sp. and 29% of ESBL-producing *E. coli* were resistant to piperacillin-tazobactam [10].

Bell and co-workers [21] found 8–50% of Klebsiella sp. resistant to piperacillin-tazobactam, while the lowest rate of resistance was reported in Japan and South Africa (8%) and the highest in Hong Kong and Singapore (50%).

Although strains with intermediate sensitivity to carbapenems among *K. pneumoniae* and *E. coli* in our institutions is still relatively low, it may indicate a higher prevalence of carbapenem resistance in the future, thereby making antibiotic therapy for these micro-organisms even more complex. Further work would be required to elucidate the mechanism of resistance before making a value judgment on its significance.

Currently, carbapenems are generally regarded as the preferred agent for treatment of infections due to ESBL-producing organisms. Newer carbapenems such as ertapenem and faropenem exhibit excellent activity against ESBL-producing organisms [22]. Although plasmid-mediated carbapenemases are unusual, chromosomally mediated, extended-spectrum serine proteases (group 2F) and metallo-β-lactamases, active against carbapenems, are not. Carbapenem resistance, due to alterations in porin proteins, has been observed in *K. pneumoniae* [22].

Due to the study design, our investigation has certain limitations: a) the clinical correlation between ESBL production and underlying disorders, hospital location and previous exposure to antibiotics was not evaluated; b) the specific clinical significance of isolating ESBL-producing organisms from non-sterile sites is still not fully known; and c) the impact of multidrug resistance on morbidity and mortality was not explored.

In summary, we described ESBL prevalence among *E. coli* and *K. pneumoniae* in two hospitals. A high prevalence of ESBL production among bacterial isolates from the adult population with a high prevalence of piperacillin-tazobactam resistance and an alarming emergence of carbapenem non-susceptible strains was found. These findings emphasize the importance of ongoing surveillance of the local epidemiology of antimicrobial resistance and the need for interventions to prevent continuous emergence of multidrug-resistant bacterial strains.

Acknowledgment. The authors wish to thank Phyllis Curchack Kornspan for her editorial and secretarial services.

References

1. Livermore DM. β-lactamase-mediated resistance and opportunities for its control. *J Antimicrob Chemother* 1998;41:25–41.
2. Garau J. Beta-lactamases: current situation and clinical importance. *Intensive Care Med* 1994;20:S5–9.

3. Menashe G, Borer A, Yagupsky P, et al. Clinical significance and impact on mortality of extended-spectrum beta lactamase-producing Enterobacteriaceae isolates in nosocomial bacteremia. *Scand J Infect Dis* 2001;33:188–93.
4. Paterson DL, Ko WC, Von Gottberg A, et al. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum beta-lactamases: implications for the clinical microbiology laboratory. *J Clin Microbiol* 2001;39(6):2206–12.
5. Colodner R, Rock W, Chazan B, et al. Risk factors for the development of extended-spectrum beta-lactamase-producing bacteria in nonhospitalized patients. *Eur J Clin Microbiol Infect Dis* 2004;23:163–7.
6. Paterson DL. Recommendation for treatment of severe infections caused by Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs). *Clin Microbiol Infect* 2000;6(9):460–3.
7. Paterson DL, Von Ko W, Gottberg A, et al. In vitro susceptibility and clinical outcome of bacteremia due to extended-spectrum beta-lactamase producing *Klebsiella pneumoniae*. *Clin Infect Dis* 1998;27:956.
8. Navov-Venezia S, Hammer-Munz O, Schwartz D, Turner D, Kuzmenko B, Carmeli Y. Occurrence and phenotypic of extended-spectrum β-lactamases among members of the family Enterobacteriaceae at the Tel-Aviv Medical Center (Israel) and evaluation of diagnostic tests. *J Clin Microbiol* 2003;41:155–8.
9. Borer A, Gilad J, Menashe G, Peled N, Reisenberg K, Schlaeffer F. Extended-spectrum beta-lactamase-producing Enterobacteriaceae strains in community-acquired bacteremia in southern Israel. *Med Sci Monit* 2002;8:44–7.
10. Schwaber MJ, Navon-Venezia S, Scwartz D, Carmeli Y. High levels of antimicrobials cross-resistance among ESBL-producing enterobacteraceae. Presented at the Annual Meeting of The Israeli Society of Infectious Diseases, 2004.
11. Colodner R, Raz R, Chazan B, Sakran W. Susceptibility pattern of extended-spectrum β-lactamase producing bacteria from inpatients to five antimicrobials drugs in a community hospital in northern Israel [Letter]. *Int J Antimicrob Agents* 2004;24(4):409–10.
12. National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard M2-A7. Villanova, PA: NCCLS, 2000.
13. Winokur PL, Canton R, Casellas JM, Legakis N. Variations in the prevalence of stains expressing an extended-spectrum β-lactamase phenotype and characterization of isolates from Europe, the Americas and the Western Pacific region. *Clin Infect Dis* 2001;32(Suppl 2):S94–103.
14. Sader HS, Jones RN, Andrade-Baiocchi S, Biedenbach DJ. SENTRY Participants Group (Latin America). Four-year evaluation of frequency of occurrence and antimicrobial susceptibility patterns of bacteria from bloodstream infections in Latin American medical centers. *Diag Microbiol Infect Dis* 2002;44:273–80.
15. Rennie RP, Jones RN, Mutnick AH. SENTRY Program study Group (North America). Occurrence and antimicrobial susceptibility patterns of pathogens isolated from skin and soft tissue infections: report of the SENTRY Antimicrobial Surveillance Program (United States and Canada, 2000) *Diagn Microbiol Infect Dis* 2003;45:287–93.
16. Du B, Long Y, Liu H, et al. Extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* bloodstream infection: risk factor and clinical outcome. *Intensive Care Med* 2002;28:1718–23.
17. The Microbiology Surveillance Network of Northern France. Surveillance of methicillin-resistant *Staphylococcus aureus* (MRSA) and enterobacteriaceae producing extended-spectrum β-lactamase (ESBL) in northern France: a five-year multicentre incidence study. *J Hosp Infect* 2002;52:107–13.
18. Jain A, Roy I, Gupta MK, Kumar M, Agarwal SK. Prevalence of extended-spectrum beta-lactamase producing Gram-negative bacteria in septicemic neonates in a tertiary care hospital. *J Med Microbiol* 2003;52:421–5.
19. Raymond J, Aujard Y. Nosocomial infections in pediatric patients: a European multicenter prospective study. European Study Group. *Infect Control Hosp Epidemiol* 2000;21:260–3.
20. Rebuck JA, Olsen KM, Fey PD, Langnas AN, Rupp ME. Characterization of an outbreak due to extended-spectrum β-lactamase producing *Klebsiella pneumoniae* in a pediatric intensive care unit transplant population. *Clin Infect Dis* 2000;31:1368–72.
21. Bell JM, Turnidge JD, Gales AC, Pfaller MA, Jones RN, for the Sentry APAC Study Group. Prevalence of extended-spectrum β-lactamase (ESBL)-producing clinical isolates in the Asia-Pacific region and South Africa: regional results from SENTRY Antimicrobial Surveillance Program (1998-99). *Diagn Microbiol Infect Dis* 2002;42:193–8.
22. Rupp ME, Fey PD. Extended spectrum β-lactamase (ESBL)-producing Enterobacteriaceae. Considerations for diagnosis, prevention and drug treatment. *Drugs* 2003;63:353–65.

Correspondence: Dr. J. Bishara, Infectious Disease Unit, Rabin Medical Center (Beilinson Campus), Petah Tiqva 49100, Israel.
Phone: (972-3) 937-6301/5
Fax: (972-3) 922-1605
email: bishara@netvision.net.il