

## FREQUENCY OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCING GRAM NEGATIVE BACILLI AMONG CLINICAL ISOLATES

RUBEENA HAFEEZ, MALEEHA ASLAM, FARZANA MIR, M. TAHIR  
IFFAT JAVAID AND AASMA N. AJMAL

*Departments of Pathology, Post Graduate Medical Institute, Allama Iqbal Medical college, and KEMU, Lahore*

### ABSTRACT

*Extended-spectrum  $\beta$ -lactamase (ESBL)-producing bacteria are emerging pathogens. They are descended by genetic mutation from native  $\beta$ -lactamases found in gram negative bacteria, especially infectious strains of *Escherichia coli* and *Klebsiella* species. Clinicians, microbiologists, infection control practitioners, and hospital epidemiologists are concerned about ESBL-producing bacteria because of the increasing incidence of such infections, the limitations of effective antimicrobial drug therapy, and adverse patient outcomes. The present study was undertaken to determine the frequency of ESBL producing gram negative bacilli recovered from clinical specimens in our setup. A total of 3099 gram negative isolates recovered from various clinical samples during the period of January 2007 to December 2008 were processed for the detection of ESBL production. Among them 35.5% bacterial strains were found to be ESBL producers. The commonest ESBL producing organism isolated was *Escherichia coli* (44.8%), followed by *Klebsiella pneumoniae* (38.6%). Production of ESBLs by gram negative bacteria is emerging as a widespread problem in our setup. Appropriate infection control and antibiotic management strategies are needed to stem the spread of this emerging form of resistance.*

**Key words:** *ESBL, frequency, Escherichia coli, Klebsiella pneumoniae, gram negative bacilli.*

### INTRODUCTION

Microbial drug resistance is an inevitable consequence of indiscriminate utilization of antimicrobial therapy<sup>1</sup>. Since we live in an era when dire predictions about the lack of effective antimicrobial drugs occur with increasing frequency, it is unclear whether we will be able to keep pace with bacterial resistance patterns and effectively treat infections in the future.<sup>2-4</sup>

Beginning with the introduction of penicillin's half a century ago, the  $\beta$ -lactams have remained the largest antibiotic class of clinical relevance. Emergence of resistance to these agents in the past two decades has resulted in a major clinical crisis.<sup>5,6</sup> Gram-negative bacteria resistant to extended spectrum cephalosporins, monobactams, carbapenems, and beta lactam beta lactamase inhibitor combinations have emerged through multiple resistance mechanisms.<sup>1-3</sup>

First described in 1980's in Europe, extended spectrum beta lactamase (ESBL) producing Enterobacteriaceae have emerged as serious nosocomial pathogens.<sup>1,5,6</sup> Recent studies have demonstrated a high prevalence of these anti-microbial resistant bacteria and also a trend of increasing resistance under continued antibiotic selective pressure<sup>4</sup>. ESBL enzymes, encoded by genes that are typically plasmid borne, hydrolyze and confer resistance to

aztreonam, cefotaxime, ceftazidime and related oxyimino-beta lactams as well as to older penicillins and cephalosporins.<sup>2,3,6</sup> Although these enzymes occur predominantly in *Klebsiella* species and *Escherichia coli*, they have been encountered in other gram negative bacilli as well.<sup>7,8</sup>

Infections with ESBL producing bacterial strains are encountered singly or in out-breaks, especially in critical care units in hospitals, resulting in increasing costs of treatment and prolonged hospital stay.<sup>4,6</sup> Prevalence of ESBL producing strains also varies from one geographical region to another paralleling the misuse or overuse of beta lactam drugs.<sup>9</sup> Despite the fact that ESBL producing organisms occur frequently, many clinicians are unaware of their existence. Due to variable affinity of these enzymes for different substrates and inoculum effect, some ESBL isolates may appear susceptible to a third generation cephalosporin in vitro.<sup>10,11</sup> Failure to detect and report antibiotic resistance due to ESBL production may result in failure to treat such infections effectively.<sup>12,13</sup>

Clinical laboratories play a pivotal role in providing accurate information and guidance in the treatment of microbial infections. Also, early identification is important in countries such as ours, where excessive use of antibiotics without antibiotic surveillance programs is the norm.

The objective of this study was to determine the frequency of ESBL producing gram negative bacilli recovered from clinical specimens in our setup.

### MATERIAL AND METHODS

Bacterial isolates included in this study were obtained from clinical samples sent for culture analysis from different hospitals of Lahore during the period of January 2007 to December 2008. A total of 3099 gram negative isolates recovered from various clinical samples of urine, blood, pus, aspirates, sputum, and bronchial secretions were processed. Study design was descriptive cross sectional and sampling technique was non probability purposive. Samples were initially inoculated on Blood and MacConkey's agar. Urine samples were cultured on Cysteine Lactose Electrolyte Deficient agar (CLED). Cultures were incubated aerobically at 37°C for 24 hours, followed by bacterial identification up to species level using API 20 E systems.

#### Screening for ESBL detection

Strains were screened using double disc diffusion technique according to the standards of NCCLS.<sup>14-17</sup> A single, separated colony of the test organism was picked and emulsified in 0.9% normal saline to match the turbidity with 0.5% McFarland's standard. The suspension was spread on Mueller Hinton

agar surface with a cotton swab soaked in the suspension. An antibiotic disc containing amoxicillin-clavulanate (20/10ug) as inhibitor of beta lactamase was placed in the center of the plate. Cefotaxime (30ug), ceftazidime (30ug), ceftriaxone (30ug) and aztreonam (30ug) discs were placed at a distance of 30 mm from the central disc as well as from each other. Zones of inhibition around the 3<sup>rd</sup> generation cephalosporin's discs and aztreonam were observed after overnight incubation at 37°C. If the inhibition zone around one or more cephalosporin's discs and aztreonam was extended on the side nearest to amoxicillin-clavulanate, the organism showing this synergism was identified as an ESBL producer. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as controls.

### RESULTS

3099 gram negative isolates were tested during the study period. These included organisms recovered from 1407 urine samples, 985 wound swabs / pus samples, 259 sputum, 219 blood, 158 body fluids and 73 bronchial washings (Table 1).

1094 (35.5%) bacterial strains were found to be ESBL producers. The commonest ESBL producing organism isolated was *Escherichia coli* (44.8%), followed by *Klebsiella pneumoniae* (38.6%), *Proteus mirabilis* (31.6%) and *Acinetobacter baumannii* (7.1%) (Table 2).

**Table 1:** Frequency of gram negative bacilli among various clinical species n = 3099.

	Specimens						
	Urine	Sputum	B. washings	Wound and pus	Fluids	Blood	Total / % age
<i>Escherichia Coli</i>	859	68	28	413	34	68	1470 / 47.4
<i>Klebsiella pneumoniae</i>	126	139	17	22	23	15	342 / 11.0
<i>Klebsiella oxytoca</i>	21	--	02	17	04	02	46 / 1.5
<i>Citrobacter freundii</i>	33	06	08	29	03	07	86 / 2.8
<i>Enterobacter cloacae</i>	29	03	06	81	12	11	142 / 4.6
<i>Proteus mirabilis</i>	129	--	--	117	04	--	250 / 8.1
<i>Proteus vulgaris</i>	89	--	--	91	09	--	189 / 6.1
<i>Acinetobacter baumannii</i>	62	24	07	128	48	63	332 / 10.7
<i>Acinetobacter spp.</i>	59	19	05	87	21	51	242 / 7.8

### DISCUSSION

Resistant bacteria are emerging as a threat to the favorable outcome of common infections in the community and hospital settings.<sup>20</sup> Beta lactamase enzymes produced by gram negative organisms confer resistance against broad spectrum beta lactam antibiotics which normally have activity against such organisms.<sup>21</sup> Recent studies

**Table 2:** Frequency of ESBL producers amongst gram negative isolates (n = 3099).

Organism isolated	No. of ESBL N = 1094	% age 35.3
Escherecia coli	659	44.8
Klebsiella pneumoniae	132	38.6
Klebsiella oxytoca	01	2.2
Citrobacter freundii	09	10.5
Enterobacter cloacae	28	19.7
Proteus mirabilis	79	31.6
Proteus vulgaris	03	1.6
Acinetobacter baumannii	78	23.5
Acinetobacter spp.	47	19.4

on ESBL production among enterobacteriaceae isolated from clinical specimens show a variable incidence ranging from as low as 1% to as high as 74%.<sup>5,6,19-20</sup> In our study, overall prevalence of ESBL production in gram negative isolates was found to be 35.5%. Shah et al<sup>25</sup> and Ali et al<sup>4</sup> have reported prevalence rates of 37.5% and 45% respectively from the northern areas of Pakistan; figures which are close to the results obtained from our area. The high percentage of ESBL producers reflects the extensive and indiscriminate use of antimicrobials in our set-up.

Klebsiella pneumoniae, Escherecia coli, Proteus mirabilis, Enterobacter spp., Citrobacter spp., Acinetobacter spp. and Pseudomonas aeruginosa are amongst the important ESBL producing gram negative bacilli reported.<sup>2,25</sup> Usually one of the three species-Klebsiella pneumoniae, Escherecia coli or Enterobacter have been reported to be as the major ESBL producers.<sup>26</sup> The prevalence of Escherecia coli and Klebsiella pneumoniae reported around the world varies greatly although it tends to be less than 50% in most studies conducted.<sup>2,1,11,16,19</sup> In our study, a prevalence of 44.8% and 38.6% was found for Escherecia coli and Klebsiella pneumoniae respectively which is similar to the results seen in other studies.

Therapeutic options available for the treatment of ESBL-associated infections are limited by drug resistance conferred by the ESBLs, along with frequently observed co-resistance to various antibiotic classes, including cephamycins, fluoroquinolones,

aminoglycosides, tetracyclines, and trimethoprim/sulfamethoxazole. In our study, amoxicillin-clavulanate, piperacillin-tazobactam, ampicillin-sulbactam, cefoxitin, imipenem, meropenem, amikacin, gentamicin and ciprofloxacin were the drugs included in the antibiotic susceptibility testing panel. Imipenem and meropenem were the only drugs to which all the isolates showed 100% susceptibility. This is in accordance with results seen in other studies.<sup>23-24</sup> The second drug showing good susceptibility (85%) in ESBL producers was Amikacin. Similar results have been reported by other researchers as well.

Relevant clinical data regarding the effectiveness of different regimens for ESBL-associated infections are limited. Although certain cephalosporins may appear active in vitro, clinical outcomes are often suboptimal. Carbapenems are regarded as the agents of choice, and may be more effective than quinolones for serious infections.

The high levels of ESBL producers mainly amongst gram negative isolates is alarming and warrants special attention, both by the clinicians and the microbiologists. In our region, testing for ESBL production is not done routinely. This may allow the undetected spread of these strains within our hospitals for long periods leading to serious outbreaks particularly in intensive care units. While the clinician has to re-evaluate the antibiotic policies, the lab must be capable of readily identifying these isolates, so that proper therapy can be instituted to avoid misuse/overuse of antibiotics.

It is **concluded** that ESBL isolates are prevalent in our setting. Routine detection of these isolates and proper control measures are recommended so that appropriate management can be instituted and spread of these organisms curtailed.

#### ACKNOWLEDGEMENTS

We are thankful to all our Lab. Staff for helping us.

#### REFERENCES

1. Jain A, Roy I, Gupta M K, Kumar M and Agarwal S K. Prevalence of extended-spectrum  $\beta$ -lactamase-producing Gram-negative bacteria in septicaemic neonates in a tertiary care hospital. *J Med Microbiol* 2003; 52: 421-25.
2. Shukla I, Tiwari R, Agrawal M. Prevalence of extended spectrum-lactamase producing Klebsiella pneumoniae in a tertiary care hospital. *Indian J Med Microbiol* 2004; 22: 87-91.
3. Subha A, Ananthan S. Extended spectrum beta lactamase (ESBL) mediated resistance to third generation cephalosporins among Klebsiella pneumoniae in Chennai. *Indian J Med Microbiol* 2002; 20: 92-95.
4. Ali AM, Rafi S, Qureshi AH. Frequency of extended spectrum beta lactamase producing gram negative bacilli among clinical isolates at clinical laboratories

- of Army Medical College, Rawalpindi. *J of Ayub Med Coll Abbottabad* 2004; 16 (1): 35-7.
5. Frank T, Arlet G, Gautier V, Talarmin A, Bercion R. Extended-spectrum  $\beta$ -Lactamase-producing Enterobacteriaceae, Central African Republic. *Emerg Infect Dis* 2006; 12 (5): 863-65.
  6. Schwaber MJ, Raney PM, Rasheed JK, Biddle JW, Williams P, McGowan Jr. JE, Tenover FC. Utility of NCCLS Guidelines for Identifying Extended-Spectrum  $\beta$ -Lactamases in Non-*Escherichia coli* and Non-*Klebsiella* spp. of Enterobacteriaceae. *J of Clin Microbiol* 2004; 42 (1): 294-98.
  7. Samaha-Kfoury JN, Araj GF. Recent developments in  $\beta$  lactamases and extended spectrum  $\beta$  lactamases. *BMJ* 2003; 327: 1209-13.
  8. Vercauteren E, Descheemaeker P, Ieven M, Sanders CC, Goossens H. Comparison of Screening Methods for Detection of Extended-Spectrum  $\beta$ -Lactamases and Their Prevalence among Blood Isolates of *Escherichia coli* and *Klebsiella* spp. in a Belgian Teaching Hospital. *J of Clin Microbiology* 1997: 2191-97.
  9. Manchanda V, Sing NP, Goyal R, Kumar A, Thukral S.S. Phenotypic characteristics of clinical isolates of *Klebsiella pneumoniae* and evaluation of available phenotypic techniques for detection of extended spectrum  $\beta$ -lactamases. *Indian J Med Res* 2005; 122: 330-37.
  10. Taneja N, Sharma M. ESBLs detection in clinical microbiology: why & how? *Indian J Med Res* 2008; 127: 297-300.
  11. Lazarova G, Rachkova K, Rukanova D, Djeneva H, Dukova I. Extended spectrum  $\beta$ -lactamases (ESBL) producing Enterobacteriaceae-challenge in the medicine practice. *Trakia J of Sciences* 2008; 6 (1): 60-63.
  12. Khurana S, Taneja N, Sharma M. Extended spectrum  $\beta$ -lactamase mediated resistance in urinary tract isolates of family Enterobacteriaceae. *Indian J Med Res* 2002; 116: 145-49.
  13. Kader AA, Kamath KA, Dass SM. Accelerated detection of extended-spectrum  $\beta$ -lactamases in clinical isolates of Enterobacteriaceae. *Br J of Biomed Sciences* 2006; 63 (4): 151-54.
  14. Performance standards for antimicrobial susceptibility testing (14<sup>th</sup> informational supplement). Approved standard. National Committee for Clinical Laboratory Standards, Wayne, PA, 2004: M100-S14.
  15. National Committee for Clinical Laboratory Standards. Performance standards for disc antimicrobial susceptibility test (7<sup>th</sup> edn). Approved standards document M2-A7. Wayne PA: NCCLS, 2000.
  16. Jain A, Mondal R. Detection of extended spectrum  $\beta$ -lactamase production in clinical isolates of *Klebsiella* spp. *Indian J Med Res* 2008; 127: 344-46.
  17. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. NCCLS approved standard. National Committee for Clinical Laboratory Standards, Wayne, PA, 2007: M100-S17.
  18. Gupta E, Mohanty S, Sood S, Dhawan B, Das BK, Kapil A. Emerging resistance to carbapenems in a tertiary care hospital in north India. *Indian J Med Res* 2006; 124: 95-98.
  19. Tankhiwale SS, Jalgaonkar SV, Ahamad S, Hassani U. Evaluation of extended spectrum beta lactamase in urinary isolates. *Indian J Med Res* 2004; 120: 553-56.
  20. Quinteros M, Radice M, Gardella N, Rodriguez MM, Costa N, Korbenfeld D, et al. Extended spectrum  $\beta$ -lactamases in Enterobacteriaceae in Buenos Aires, Argentina, Public Hospitals. *Antimicrob Agents Chemother* 2003; 47: 2864-67.
  21. Shah AA, Hasan F, Ahmed S, Hameed A. Prevalence of extended spectrum  $\beta$ -lactamases in nosocomial and outpatients (ambulatory). *Pak J Med Sci* 2003; 19 (3): 187-91.
  22. Grover SS, Sharma M, Pasha ST, Singh G, Lal S. Antimicrobial susceptibility pattern and prevalence of extended spectrum beta-lactamase (ESBLs) producing strains of *Klebsiella pneumoniae* from a major hospital In New Delhi. *J Commun Dis* 2004; 36 (1): 17-26.
  23. Luzzaro F, Mezzatesta M, Mugnaioli C, Perilli M, Stefani S, Amicosante G, Rossolini GM, Toniolo A. Trends in Production of Extended-Spectrum  $\beta$ -Lactams among Enterobacteria of Medical Interest: Report of the Second Italian Nationwide Survey. *J of Clin Microbiol* 2006; 44 (5): 1659-64.
  24. Romanus II, Egwu OA, Ngozi AT, Chidiebube NA, Chika EP. Extended Spectrum Beta – Lactamase (ESBL) Mediated Resistance to Antibiotics Among *Klebsiella Pneumoniae* in Enugu Metropolis. *Maced J Med Sci* 2009; 15; 2 (3): XX-XX. (Epub ahead of print).
  25. Malhotra VL, Khandpur N, Dass A, Mehta G. Prevalence of extended spectrum beta lactamases producing clinical isolates from patients of urinary tract infection in a tertiary care hospital in Delhi. *J Commun Dis* 2008; 40 (4): 269-72.
  26. Ko WC, Hsueh PR. Increasing extended-spectrum beta-lactamase production and quinolone resistance among Gram-negative bacilli causing intra-abdominal infections in the Asia/Pacific region: Data from the Smart Study 2002-2006. *J Infect.* 2009; XX\_XX. [Epub ahead of print].
  27. Ahmad S, Al-Juaid NF, Alenzi FQ, Mattar EH, Bakheet OE. Prevalence, Antibiotic Susceptibility Pattern and Production of Extended-Spectrum beta-Lactamases Amongst Clinical Isolates of *Klebsiella pneumoniae* at Armed Forces Hospital in Saudi Arabia. *J of College of Physicians and Surgeons Pakistan* 2009; 19 (4): 264-65.
  28. Aladag MO, Durak Y. Investigations of some antibiotic susceptibilities, plasmid profiles and ESBL characteristics of *Klebsiella pneumoniae* isolated from urinary system infections. *World Appl Sc. J* 2009; 6 (5): 630-36.