Prevalence of Extended spectrum beta lactamase (ESBL) production in gram negative isolates from pyogenic infection in tertiary care hospital of Eastern Nepal

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ABSTRACT

Emergence of extended spectrum beta lactamase (ESBL) producing isolates has important clinical and therapeutic implications. A high prevalence of ESBL production among multidrug resistant gram negative isolates has been reported in literature from various clinical samples. Since ESBL detection is not done on a routine basis, its prevalence is not known till date. Thus the present study was undertaken to determine the prevalence of ESBL production in gram negative isolates from pyogenic infection. A total of 300 gram negative bacilli isolated from the pus samples were identified phenotypically and antimicrobial activity was determined. ESBL detection among the isolated organisms was done by Phenotypic confirmatory disc diffusion technique recommended by CLSI. Of the 300 isolates, majority were *Escherichia coli;* (107) followed by *Acinetobacter* species; (55), *Pseudomonas* species; (44)*, Klebsiella pneumoniae;* (32)*, Proteus mirabilis;* (26), *Enterobacter s*pecies; (25), *Citrobacter* species; (9) and others; (2). The prevalence of ESBL producing organisms was found to be 54 (18%); amongst which *Escherichia coli* was 29 (53.7%), *Klebsiella pneumonia* (14.8%), *Proteus mirabilis* 7 (12.9%) and others 4 (7.4%). Multidrug resistance were found in 92.6% of ESBL producers. Forty two were resistant to all the three third generation cephalosporins. The continuous surveillance of the ESBL producing isolates is necessary to make aware about the correct treatment regimens and good infection control practices.

Keywords: ESBL, Gram negative bacilli.

INTRODUCTION

Extended spectrum beta lactamases (ESBLs) are enzymes which are derived from TEM or SHV (class A) enzymes. They confer variable levels of resistance to cefotaxime, ceftazidime and other broad-spectrum cephalosporins and to monobactams such as aztreonam.[1]

Emergence of ESBL-producing isolates has important clinical and therapeutic implications. In most bacterial isolates, resistance determinants for extended spectrum beta lactamase (ESBL) production are carried on plasmids that can be easily spread from organism to organism and the spread of resistance towards extended-spectrum cephalosporins further restricts the use of beta-lactam antibiotics and may lead to increased prescription of more broad-spectrum and expensive antibiotics such as Imipenem.[2] In addition, these resistant isolates may escape detection with routine in vitro susceptibility testing performed by a clinical microbiology laboratory, which can result in adverse therapeutic outcomes.[3,4] More importantly, antibiotic selection for treatment of serious infections due to ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* is a clinical challenge due to the complex nature of in vitro susceptibility testing and in vivo correlation. Perhaps the biggest challenge lies in overcoming widespread unawareness among clinicians regarding these resistant organisms due to under reporting by microbiology laboratories and lack of an obvious marker to indicate production of an ESBL.[5]

Nosocomial infections due to ESBL producing organisms have been known to cause high mortality.[6] It is of utmost importance to know the prevalence of ESBL producing organisms in a hospital to formulate a policy of empirical therapy in high risk areas where infections due to resistant organisms is much higher.[7] Similarly, it helps to avoid misuse of extended spectrum cephalosporins, which remains drug of choice in most of the hospitals.[7]

The aim of this study was to determine the prevalence of ESBL producing gram negative bacilli isolated from pus samples in the Microbiology laboratory of BPKIHS.

MATERIALS AND METHODS

The study was carried out in the Clinical laboratory Service, Department of Microbiology, B. P. Koirala Institute of Health Sciences, Dharan, Nepal, a tertiary care centre, from June 2006 to May 2007. A total of 300 gram negative bacilli (identified on the basis of colony characters, gram stain, biochemical properties) isolated

Table-1: Distribution of different ESBL producing organisms

Organisms	Total Isolates	ESBL Producing strains n. (%)
Escherichia coli	107	29 (27.10)
Acinetobacter spp	55	3 (5.45)
Pseudomonas spp	44	1 (2.3)
Kleb pnuemoniae	32	8 (25)
Proteus mirabilis	26	7 (26.92)
Enterobacter spp	25	3 (12)
Citrobacter spp	9	3 (33.33)
Serratia marcescens	1	0 (0)
Morganella morganii	1	0 (0)

from the pus specimen obtained from various parts of the hospital were included in the study for evaluation of ESBL property. The antimicrobial sensitivity test was performed by Kirby Bauer disc diffusion technique using ampicillin (30mg), ceftazidime (30mg), cefotaxime (30mg), ciprofloxacin (5mg), ceftriaxone (30mg) and gentamicin (10mg). Additional disc like carbenicillin (100mg), tobramycin (10mg), piperacillin (100mg) were used for *Pseudomonas* species. As control strain *E. coli* ATCC 25922 was used. Diameter of the zone of inhibition for each antibiotic was measured and interpreted according to CLSI guidelines.

Detection of ESBLs: All gram negative bacilli were tested for ESBL production by Phenotypic Confirmatory disc diffusion test method recommended by CLSI. The medium used was Mueller-Hinton agar and the antibiotics used were ceftazidime (30mg) and ceftazidime/clavulanic acid (30/10mg). Organism was considered as ESBL producer if there was a > 5mm increase in zone diameter of ceftazidime/clavulanic acid disc than that of ceftazidime disc alone. Positive control of *K. pneumoniae* ATCC 700603 and negative control of *E. coli* ATCC 25922 were also inoculated in the separate culture plate along with the organism to be tested.

Table-2: Distribution of ESBL producing organisms in different wards

Wards	No of total isolates	ESBL producing strains
		n. (%)
Surgery	154	35 (22.73)
Orthopedics	20	3 (15)
Emergency	19	1 (5.26)
Medicine	12	2 (16.67)
Gynaecology	10	3 (33.33)
Paediatrics	7	1 (28.57)
ENT	6	1 (16.66)
ICU	4	1 (25)
Opthalmology	1	1 (100)

RESULTS

Out of 300 isolates, 236 belonged to inpatient and 64 to outpatient. The most common isolates were *E. coli* (107) followed by *Acinetobacter spp* (55) and *Pseudomonas spp* (44) (Table-1). Two-third (66.3%) of isolates was multidrug resistant. Antimicrobial resistance pattern of Gram Negative isolates is shown in Fig. 1.

ESBL production: Out of 300 gram negative bacilli, 54 (18.0%) were ESBL producers out of which 49 (91.0%) were from inpatients and 5 (9.0%) from outpatients. Frequency of ESBL producers and their percentage in different isolates are shown in Table 1. On studying the ESBL producing strain in relation to the age group of the patients, the distribution was observed to be almost similar in all the age groups, the range being 15.9%-20.5%. Distribution of ESBL producers from various wards is shown in Table-2. Maximum number of ESBL producers were from clinical cases of post operative wound infection (Table-3).

Multidrug resistance was seen in 92.6% of ESBL producers; of these, 29(58.0%) strains were identified as *E. coli*, 8(16.0%) as *K pneumoniae*, 6(12.0%) as *Proteus mirabilis*, 2(4.0%) each as *Enterobacter spp*, *Citrobacter spp* and *Pseudomonas spp.* Majority of isolates, 42 (77.7%) ESBL producers were resistant to all three third-generation cephalosporins tested. Only 4(7.0%) isolates were susceptible to cefotaxime, 1 to ceftriaxone and 6 (11.0%) to both (Table-4). All the isolates were resistant to ampicillin and ceftazidime whereas 35 (65.0%) were resistant to ciprofloxacin and 38 (70.0%) to gentamicin.

DISCUSSION

Over the past decade, ESBL producing Enterobacteriaceae have emerged as serious nosocomial pathogens throughout the world.[8] In the United States, the occurrence of ESBL ranges from 0-25% and the national average is 3%.[8] In India, the prevalence rate varies in different institutions from 12.6%-66.7%.[8-16] In the present study, 54 (18.0%) were found to be ESBL producers which is in the lower range of the spectrum.

In the present study, 27.1% of *E. coli* were ESBL producers which is comparable to the study by Shah *et al*[12] but reported much higher in other studies.[8,11,12] Although *Acinetobacter spp*., known to be a nosocomial

Table-3: Correlation of different ESBL producers with clinical presentation

Clinical history	Total no of cases	Esch coli	Klebsiella pneumonia	Proteus mirabilis	Enterobacter spp	Acinetobacter Spp	Citrobacter spp	Pseudomonas spp
P/PP *	6	4	1	0	1	0	0	0
POWI**	16	9	3	2	0	2	0	0
Abscess	3	2	0	0	0	0	0	1
Burn	4	1	0	1	1	0	1	0
Injury/trauma	4	1	0	3	0	0	0	0
DM***	3	2	1	0	0	0	0	0
Pyelonephritis	2	2	0	0	0	0	0	0
Miscellaneous	11	5	2	0	1	1	2	0
Total no of org	49	26	7	6	3	3	3	1

*P/PP: Peritonitis/Perforation peritonitis, **POWI: Post operative wound infection, ***DM: Diabetes mellitus

pathogen, was second highest organism isolated in the present study, ESBL production was seen in only 5.4% which is very low in comparison with the other studies which had 72.0% and 66.7%.[11] Only one isolate of *Pseudomonas spp*. (2.3%) was ESBL producer in the present study which is very less on comparison with the study conducted by Mohanty *et al*.[11] In the present study, 25.0% of the *K. pneumoniae* were found to be ESBL producers which is slightly higher to that reported by David *et al*.[17] Other workers have reported a high percentage of ESBL producing *K. pneumoniae* varying from 57.0-70.0%.[8,14] David *et al*[17] had conducted a prospective study spanning over 12 hospitals in South Africa, Taiwan, Australia, Argentina, the United States, Belgium, and Turkey and had studied ESBL production only in *K. pneumoniae*. Their publication also highlights a high variation in the ESBL producing *K. pneumoniae* episodes of nosocomial bacteremia varying from 7% in Taiwan to 78% in Turkey. 26.92% of Proteus mirabilis were found to be ESBL producers in the present study which is comparable with 30% reported by Shah et al and much lower than reported by Ali AM[8](61%). Similarly only 12% of *Enterobacter spp.* in the present study was observed to be ESBL producers compared to 33.33% -79% respectively by other authors.[8,11,12] ESBL production in *Citrobacter spp.* was detected in only 33.33% of isolates compared to 67.5% reported by Mohanty et al[11]. No ESBL production was detected in *Serratia marcescens* and *Morganella morganii* in the present study.

BL producing organisms are responsible for a variety of infections.[18] In correlation with the clinical presentation, maximum number of ESBL producers were from the pus samples obtained from post operative and other wound infections followed by peritonitis/perforation peritonitis, burn, injury/trauma, infections related to diabetes mellitus and abscesses showing ESBL producing organisms responsible for causing variety of infections. *E. coli* was the predominating organism causing most of the infections. Distribution of ESBL producers were almost similar in all age groups. As pus was the only sample included in the present study, the maximum number of ESBL producers were from the department of surgery. No similar correlation have been reviewed in the other literature.

Multidrug resistance is expected to be more common in ESBL producing organisms as the transfer of gene is

Table-4: The resistance pattern of ESBL producing organisms to 3rd generation cephalosporins (3GC)

SN	Combination of 3GC	Number of Esbl producers
1	Ca Ce Ci	42
2	Ca Ci	4
3	Ca Ce	1
4	Ca	6

Ca : Ceftazidime, Ce : Ceftriaxone, Ci : Ceftriaxone

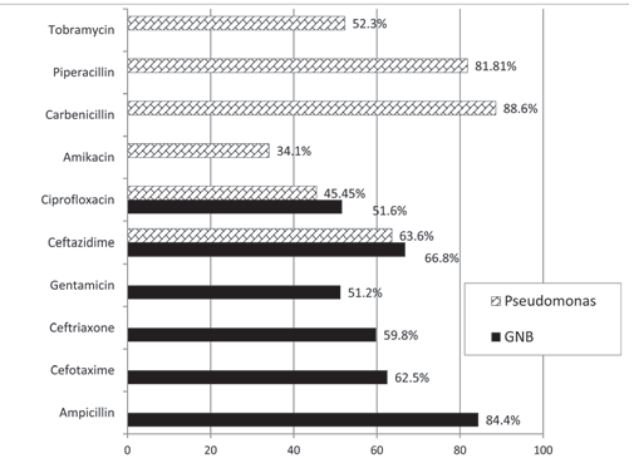

Fig. 1. Resistance pattern of Gram negative isolates to different antibiotics

plasmid mediated. In the present study, all the ESBL producers were resistant at least to one of the four classes of antibiotics tested. 50 out of 54 (92.5%) were multidrug resistant which is similar to the prevalence of 90.5% multidrug resistance ESBL producers in the study carried out by Supriya *et al*.[15] Co-resistance of ESBL producing organisms to quinolones and aminoglycosides are common.[18,19] 64.8% of ESBL producers were resistant to ciprofloxacin and 70.3% resistant to gentamicin.

Extensive use of 3rd generation cephalosporins has contributed to the evolution of ESBL.[17] All 54 ESBL producers in the present study were resistant to ceftazidime whereas Shukla et al[13] had reported 6.2% of the ESBL producers sensitive to ceftazidime. The number of strains susceptible to cefotaxime and ceftriaxone in the in vitro disk diffusion test in the present study is comparable to that reported by Shukla et al[13]; the percentage being 18.5% and 18.7% respectively for cefotaxime and 12.9% and 9.5% respectively for ceftriaxone. ESBLs constitute a serious threat to current beta-lactams. As per CLSI guidelines, any strain which is identified as ESBL producers should be reported as resistant to all the 3rd generation cephalosporin irrespective of its resistance or susceptible status in the disc diffusion test which makes treatment to ESBL infections difficult.[20] In the present study, 11 out of 54 (20.3%) strains were observed to be susceptible to cefotaxime and/or ceftriaxone which gains importance from the point of view of management of cases specially so as majority of the patients in the present study were admitted mainly in the surgical wards where these drugs were given as the standard periopeative antibiotics.

Since most of the isolates showed multidrug resistance, the therapeutic strategies to control infections due to ESBL producing organisms has to be carefully formulated. The therapeutic use of all 3rd generation cephalosporins should be avoided if an organism appear resistant to any one of them and are ESBL producers.

REFERENCES

1. Chow JW, Fine MJ, Shlaes DM. Enterobacter bacteremia: clinical features and emergence of antibiotic resistance during therapy. *Ann Intern Med* 1991; 115: 585-90.
2. Livermore DM. ß-Lactamases in laboratory and clinical resistance. *Clin Microbiol Rev* 1995; 8: 557-84.
3. Bush K. Is it important to identify extended-spectrum ß-lactamase-producing isolates? *Eur J Clin Microbiol Infect Dis* 1996; 15: 361-4.
4. Tenover FC, Mohammed MJ, Gorton TS, Dembek ZF. Detection and reporting of organisms producing extended-spectrum ß-lactamases: survey of laboratories in Connecticut. *J Clin Microbiol* 1999; 37: 4065-70.
5. Paterson DL, Yu VL. Extended-spectrum ß-lactamases: a call for improved detection and control. *Clin Infect Dis* 1999; 29: 1419-22.
6. Ho PL, Chan WM, Tsang KW, Wong SS, Young K. Bacteremia caused by Escherichia coli producing extended-spectrum beta-lactamase: a case-control study of risk factors and outcomes. *Scand J Infect Dis*. 2002; 34: 567–73.
7. Mathur P, Kapil A, Das B, Dhawan B. Prevalence of ESBL producing gram negative bacteria in a tertiary care hospital. *Indian J Med Res* 2002; 115: 153-57.
8. Ali AM, Rafi S, Qureshi AH. Frequency of extended spectrum beta lactamases producing gram negative bacilli among clinical isolates at clinical laboratories of Army Medical College, Rawalpindi, Pakistan. www.ayubmed.edu.pk/JAMC/PAST/16-1/Aarif.htm
9. Nduguilile F, Jureen R, Harthug S, Urassa W, Langeland N. Extended Spectrum Beta Lactamases among Gram negative bacteria of nosocomial origin from an Intensive Care Unit of a tertiary health facility in Tanzania. *BMC Infect Dis* 2005; 5: 86.
10. Datta P, Thakur A, Mishra B, Gupta V. Prevalence of Clinical Strains Resistant to Various beta lactams in a Tertiary Care Hospital in India. *Jpn. J. Infect. Dis*. 2004; 57: 146-9.
11. Mohanty S, Kapil A, Dhawan B, Das BK. Bacteriological and antimicrobial susceptibility profile of soft tissue infections from Northern India. *Indian J Med Sci* 2004; 58: 1.
12. Shah AA, Hasan F, Ahmed S & Hameed A. Prevalence of Extended spectrum beta lactamase in Nosocomial and Outpatient (Ambulatory), *Pakistan J Med Sci* 2000; 19: 187-91.
13. Shukla I, Tiwari R, Agrawal M. Prevalence of extended spectrum -lactamase producing K*lebsiella pneumoniae* in a tertiary care hospital. *Indian J Med Microbiology* 2004; 22: 87-91.
14. Duttaroy B, Mehta S. Extended spectrum beta lactamases (ESBL) in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli*. *Indian J Pathol Microbiol* 2005; 48: 45-8.
15. Tankhiwale SS, Jalgaonkar SV, Ahamad S, Hassani U. Evaluation of extended spectrum beta lactamase in urinary isolates. *Indian J Med Res*. 2004; 120: 553-6.
16. Kumar MS, Lakshmi V, Rajagopalan R. Occurrence of extended spectrum beta lactamases among Enterobacteriaceae spp. isolated at a tertiary care institute. *Indian J Med Microbiol* 2006; 24: 208-11.
17. Menon T, Bindu D, Kumar CPG, Nalini S, Thirunarayan MA. Comparison of double disc and three dimensional methods to screen for ESBL producers in a tertiary care hospital. *Indian J Med Microbiol*. 2006; 24: 117-120.
18. Bhattacharya S. ESBL- From Petri Dish to the Patient. *Indian J Med Microbiol* 2006; 24: 20-24.
19. Dancer SJ. Extended Spectrum Beta lactamases- are we prepared to face the threat? SCIEH Weekly Report. 2004; 38: 50.
20. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. M100-S16. Sixteenth informational supplement. Wayne, PA: CLSI; 2006.