



## Prevalence of Multidrug Resistant Extended-Spectrum Beta-Lactamase Producing Gram-Negative Bacteria in Neonatal Sepsis

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### Abstract

**Objectives:** Neonatal sepsis with extended-spectrum beta-lactamase (ESBL) producing microorganisms is recognized increasingly in recent years. ESBL can be produced by various bacterial strains. This study was conducted to determine the prevalence of ESBL producing pathogens in neonatal sepsis and its impact on clinical outcome.

**Materials and Methods:** A study was carried out from Jan 2012 to Jan 2013 in a referral university hospital. All neonates who had diagnosed as sepsis were enrolled in this study. Blood cultures were processed using the automated BACTEC 9120 system. Antibiotic resistance and beta-lactamase production of bacterial isolates was tested. All patients were followed till discharge.

**Results:** : One hundred three neonates with gestation age  $36.7 \pm 3.2$  weeks were enrolled in this study and 56 cases (54%) were boys. The most common isolated gram negative pathogens were Klebsiella pneumoniae, Acinetobacter species, and Pseudomonas aeruginosa. The rate for beta-lactamase production were 97.7% in Klebsiella pneumoniae, 81.3% in Acinetobacter, 85.7% in E. coli, 53.3% in Pseudomonas aeruginosa and 100% in Serratia. Thirty eight (35.9%) neonates were dead, that 34 of them were beta-lactamase producers. The mean duration of hospitalization were longer in patients infected with beta-lactamase producers ( $30.2 \pm 20.5$  vs.  $22.8 \pm 16.6$  days  $P=0.05$ ) and ESBL producing strains ( $29.13 \pm 20.39$  vs.  $19 \pm 9.84$   $P=0.05$ ). ESBL production rate were determined 95.5% and 86.7% in Klebsiella pneumoniae by combined disk test (CDT) and double disk synergy test (DDST) method, respectively. These methods were positive for ESBL production in 78.6% and 64.3% of E. coli isolates, respectively. **Conclusion:** in our study, the high rate of beta-lactamase and ESBL production were determined for common isolated organisms in neonatal sepsis. Infection with ESBL producing pathogens was associated with longer hospital stay. CDT method was detected more ESBL producing pathogens than DDST method in our study. It is recommended future studies to determine the risk factors predisposing newborn infants with these pathogens.

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## Introduction:

Sepsis is a major cause of hospital admission and mortality in newborn infants. The mean age at onset of first episode of late onset sepsis is 2-3 weeks, independent to the infecting pathogen. Antibiotic resistance is a serious concern in management of neonatal sepsis. Sepsis with extended-spectrum beta-lactamase (ESBL) producing microorganisms is recognized increasingly in Asia countries in recent years (1-3). ESBL producing organisms were first isolated in Germany in 1983 (4), then in France, United States and Asia (5). ESBL is recognized worldwide as a problem in hospitalized neonates. The prevalence of ESBL among clinical isolates varies between countries and hospitals.

ESBL can be produced by various bacterial strains mainly *E. coli*, *Klebsiella pneumoniae*, *Citrobacter*, *Proteus*, *Salmonella* and *Shigella* species (4, 5). ESBLs are plasmid mediated enzymes that hydrolyze broad spectrum beta-lactamase (6). Risk factors associated with infection caused by ESBL producing agents include central venous catheters, tracheostomy and cephalosporin use (7, 8). Indiscriminate use of antibiotics can result in colonization or infection with drug resistant strains of bacteria including ESBL-producing pathogens. Emergence of extended spectrum beta-lactamase producing strains of gram negative bacteria often complicates the clinical and therapeutic outcome of neonatal sepsis (9).

It is necessary to have a surveillance of antibiotic resistance, limit the antibiotic use and have an appropriate antibiotic prescription practice for reducing the emergence and spread of antimicrobial resistance and updating guidelines of empirical antibiotic therapy. There are a few studies about the prevalence of ESBL producing pathogens in neonatal sepsis in Iran. We conducted this study to determine the proportion of isolates producing ESBLs and its impact on clinical outcome in neonatal sepsis.

## Material & Methods:

A prospective descriptive analytic study was carried out from January 2012 to January

2013 at the Tabriz Children's hospital, which is a referral university hospital in the northwest of Iran. The study was approved by the ethic committee of the university.

All patients who had clinical manifestations of sepsis, including thermal instability, hypotension, tachycardia, tachypnea and leukocytosis or leucopenia, were eligible for inclusion in the study. They were enrolled in study if the diagnosis of sepsis was confirmed with positive blood culture, urine culture obtained in a sterile manner, cerebrospinal fluid culture or additional cultures obtained as indicated by clinical findings. Demographic characteristics of patients and results of their laboratory tests were collected. All patients were followed till discharge.

Standard methods were used for the analysis and culture of clinical specimens. Blood cultures were processed using the automated BACTEC 9120 system (BD Diagnostic). All gram negative isolates were identified on the basis of their colony, morphology, culture characteristics, and their biochemical reactions according to routine bacteriologic procedures (10). Repeat isolates were excluded. These were defined as an isolate that was the same organism with the same susceptibility profile and from the same patient at different times.

The antibiotic susceptibility of bacterial isolates was performed by the disc diffusion method (Kirby-Bauer) according to the CLSI recommendations (11).

The antibiotic disks used were amikacin, gentamicin, ciprofloxacin, co-trimoxazole, imipenem, chloramphenicol, ceftriaxone, ceftizoxime, ceftazidime and cefixime (Mast Co, UK). For statistical analysis, bacteria with intermediate susceptibility were considered resistant. Multidrug resistance was considered if the isolate showed resistance to more than two unrelated drugs.

Bacterial isolates were tested for  $\beta$ -lactamase production by the nitrocefin test 12. Briefly, Cefinase disks impregnated with nitrocefin (Mast Co, UK), moistened with one drop of water and several well-isolated similar colonies are smeared onto the disk surface, and observed up to 1 hour. If the

bacterium produces  $\beta$ -lactamase in significant quantities, the yellow-colored disk turns red.

To detect ESBL production, double disk synergy test (DDST) and combined disk test (CDT) was performed using cation-adjusted Mueller-Hinton agar (Becton-Dickinson, Sparks, MD, USA) as described in previous studies 13, 14.

Statistical analyses were performed using descriptive statistics such as frequency, percentage, mean and standard deviation. The association of variables was compared by using Chi-square or Fisher's exact test as appropriate. The significance level was defined as  $P < 0.05$ .

### Results:

Total 103 neonates were enrolled in this study consisting 56 boys (54%) and 47 girls (46%). The mean gestation age of studied patients was  $36.7 \pm 3.2$  weeks and birth weight  $2650 \pm 612$  grams. The mean post-natal age of neonates at hospital admission was  $6.1 \pm 2.3$  days. Pathogens were isolated from blood culture in 86 cases, urine 13 patients, CSF in 2 cases, peritoneal aspirate in one patient and middle ear aspirate in one case. Fifty eight patients have underwent surgical procedure including esophageal atresia (18 cases), intestinal obstruction (16 cases), diaphragmatic hernia (9 patients) and choanal atresia (6 neonates). The most common isolated pathogens were *Klebsiella pneumoniae* (N= 45), *Acinetobacter* species (N = 16) *Pseudomonas aeruginosa* (N = 15), *E. coli* (N= 14) and remaining were *Serratia* species, *Stenotrophomonas maltophilia*, and non- *aeruginosa Pseudomonas* species.

The mortality rate was 35.9% (thirty eight neonates) that 16 cases were girls ( $P=0.61$ ). Among these patients 17 neonates were delivered vaginally ( $P=0.97$ ) and 24 patients had surgical operative procedure ( $P=0.37$ ).

The demographic and laboratory characteristics of patients were compared according to their outcome and showed in table 1.

Antimicrobial resistance patterns of major isolated pathogens were determined and showed in graph 1.

Eighty seven (84.5%) isolates were beta-lactamase producer. ESBL production was found in sixty eight isolates (66%) with CDT method and 66 isolates (64.1%) with DDST method. The beta-lactamase and ESBL production of different pathogens showed in graph 2.

The mortality rate among beta-lactamase producing pathogens was 34/87 (39.1%), and this indicates that 34 cases of expired neonates were beta lactamase producer. It was 29/68 (42.6%),  $p=0.07$  in CDT positive species and 28/66 (42.4%) in DDST positive pathogens,  $p=0.09$ . The duration of hospitalization was compared in patients infected with beta-lactamase and ESBL producing pathogens and showed in table 2.

### Discussion:

ESBL producing organisms represent an emerging infectious threat. As the study by Kuhmar (15), the most common isolated pathogen was *Klebsiella pneumoniae* in our study. Ninety seven percent of isolated *Klebsiella* species were beta-lactamase producer and resistant to third generation cephalosporins that is in agree to study of Jain et al (16).

The rate of ESBL producing *Escherichia coli*, *Klebsiella pneumoniae* and *oxytoca*, between Jan 2003 and Dec 2007, was reported by Blashke that increased from 0.53% to 1.4% (17). In this study, we showed a significant frequency of ESBL producing pathogens in neonatal sepsis. ESBL producing *E. coli* strains was over 64% in our study.

The most common multi drug resistant organism in our study was *E. coli* followed by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Intensive care unit admission, prolonged hospitalization, use of antimicrobials and use of medical devices are major known risk factors for ESBL acquisition in hospital based pathogens (18, 19). Over 50% of our studied patients had NICU admission because of surgical repair for congenital malformations, had endotracheal intubation, mechanical ventilation and received broad spectrum antibiotics. Use of antimicrobial drugs particularly third generation cephalosporins is a major cause favouring the emergence of

resistance organisms including ESBL producing *Klebsiella pneumoniae* and *E. coli*. The studied patients developed sepsis approximately one week after hospitalization.

Thaver reported a high rate of ampicillin and co-trimoxazole resistance among the three major isolated pathogens in neonatal infections in developing countries including *E. coli*, *Staphylococcus aureus* and *Klebsiella* species (20). They reported resistance to third generation cephalosporins 19%, ampicillin 72% and co-trimoxazole 78% in *E. coli*. In our study, resistance to third generation cephalosporins and cotrimoxazole was 70% and 42.9% respectively. Among *Klebsiella* species, antibiotic resistance was 45% to co-trimoxazole and 66% to third generation cephalosporins in Thaver study. In our study, among *klebsiella* species, resistance rate were 36.9% and 88% to co-trimoxazole and third generation cephalosporins respectively. Reported mortality rates in neonatal sepsis are as low as 10%, but several studies documented that sepsis case fatality rate is highest for gram negative and fungal infections. Multidrug-resistant gram-negative pathogens are alarming and increase the risk of adverse outcomes including prolonged hospitalization and mortality. In our study, mortality rate was 36.9% and 89% of them were infected by beta-lactamase producing pathogens. As it is showed in table 1, neonates with lower birth weight and gestation age had higher mortality rate in neonatal sepsis. The duration of hospitalization was longer in patients infected by beta-lactamase producing gram-negative pathogens. ESBL producing pathogens were determined by CDT method more than DDST method.

### **Conclusion :**

There was high prevalence of multidrug-resistant gram-negative pathogens in neonatal sepsis and high mortality rate in our study. High rate of beta-lactamase and ESBL production were also determined in this study. Most ESBL producing pathogens were determined by CDT method in our study. It is recommended future studies to determine the risk factors for infection with

ESBL producing pathogens. It is recommended future studies with implementation an antimicrobial formularity intervention to restrict the use of third generation cephalosporins to assess the effect of these interventions on incidence of multidrug-resistant gram-negative bacteria. The rational use of antibiotics in neonates involves using narrow spectrum drugs when possible, treating infection and not colonization, and limiting the duration of therapy. Regarding to the lack of significant differences between the two groups, further studies with more number of cases is recommended for better clearance of the topic.

### **Conflicts of interest:**

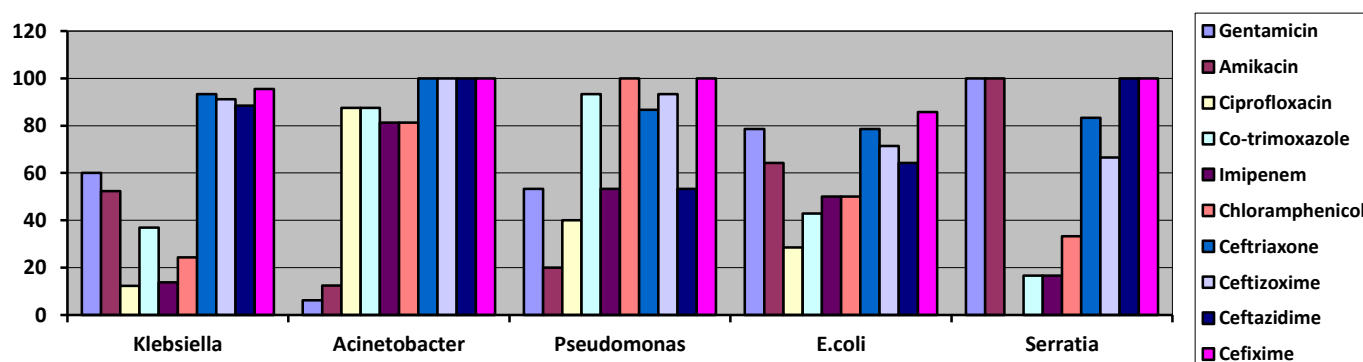
The authors declare no conflict of interest in this study.

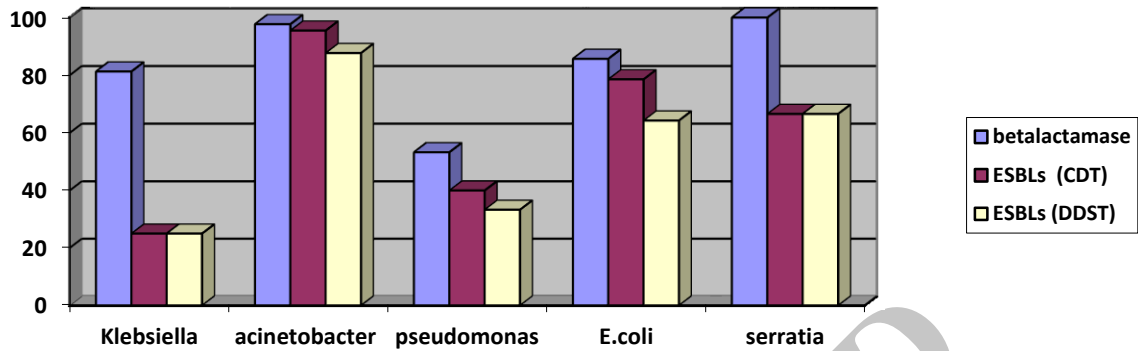
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**Table 1.** Demographic characteristics and laboratory tests in patients according their outcome.

	Discharged N=63	Expired N=38	P value
Birth weight (g)	2840 ±476	2350±692	< 0.001
Gestation age (wk)	37.8±2.3	35.1±3.7	< 0.001
Gender, male, n (%)	32 (50.8)	22 (57.9/)	0.61
Route of delivery NVD, n (%)	31 (49.2)	17 (44.7)	0.97
Age at admission (d)	5±0.8	7.7±1.4	0.09
Age of sepsis (d)	12.5±1.5	17.8±4.3	0.18
White blood cell count	13159±805	16828±1212	0.09
Platelet count	220980±17200	118260±33000	0.01
Haemoglobin (g/dl)	13.7±3.3	11.9±3.2	0.01
C reactive protein	1±0.1	1.5±0.2	0.06

**Graph 1.** Antimicrobial resistance of pathogens.

**Graph 2 .** Prevalence of beta-lactamase and ESBL production in different pathogens.**Table 2.** Hospital stays in septic cases according to their beta-lactamase patterns .

	Hospital stay, days mean±SD	P value
CDT		
Positive	29.32±20.92	0.22
Negative	24.16±18.11	
DDST		
Positive	29.13±20.39	0.05
Negative	19±9.84	
β-lactamase		
Positive	30.2±20.5	0.05
Negative	22.8±16.6	

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