Original Research Article

Incidence of ESBL Producing Organisms In Neonatal Sepsis

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Abstract

Introduction: In neonates (0–28 days) ESBL-producing Klebsiella pneumoniae is an important cause of nosocomial infections. The present study is directed to determine the frequency of infections caused by ESBL-producing organisms, the various bacteria producing ESBL, and the antibiotic susceptibility of these organisms in a neonatal intensive care unit in a tertiary care hospital of Bihar. **Methodology:** This study was conducted in the tertiary-level referral and inborn neonatal units by the Department of Microbiology at GMC, Bettiah, Bihar, India, from March 2020 February 2021. Written informed consent was obtained for each subject from the parents. The study was approved by the institute's Ethics Committee. All patients with suspected neonatal sepsis admitted during the study period were included in the study. A total of 120 cases were enrolled in the study during the study period. Data was analyzed using Statistical Package for Social Sciences ver. 20.0 (IBM, Chicago). **Results:** Culture-positive gram-negative organisms were found in 42 neonates. Of these mixture of organisms was found in 5 neonates and was excluded from the study. The incidence of ESBL producing organism was 5%. Klebsiella was isolated from 4 out of 6 ESBL positive samples and another 2 yielded Escherichia coli. Among the 31 ESBL negative culture, majority were Acenitobacter (51.6%) followed by Klebsiella (29%) and then Enterobacter (12.9%). **Conclusion:** Longitudinal surveillance of the microbial flora and their antibiotic sensitivity pattern should be done in every hospital periodically to know the existing flora and for appropriate management of the infection by these organisms.

Key Words: Neonatal Sepsis, ESBL Producing Organisms

Introduction

Broad spectrum penicillins and first-generation cephalosporins remained the first line of defense for nearly 20 years, before resistance to them by beta-lactamases produced by gram-negative bacilli was found to be a serious threat to the common infections prevalent in community and hospital settings[1]. In a few years, cephalosporin-resistant Klebsiella species were found among the clinical isolates and the mechanism of this resistance was the production of extended-spectrum beta-lactamase (ESBL)[2].The first ESBL isolates were discovered in Western Europe in the mid-1980s[3]. ESBLs are plasmid mediated beta-lactamases capable of hydrolyzing and inactivating extended spectrum beta-lactams with an oxyimino side chain like cephalosporins (cefotaxime, ceftriaxone, and ceftazidime) and oxyimino-monobactam (aztreonam). They have no detectable activity against cephamycins and carbapenems[4]. ESBLs are most commonly found in Klebsiella species and Escherichia coli. But they have also been detected in Enterobacter species, Salmonella species, Morganella morganii, Proteus mirabilis and Pseudomonas aeruginosa[5].Major risk factors for infection with ESBL-producing organisms are widespread use of third-generation cephalosporins, prolonged intensive care unit (ICU) or hospital stay, instrumentation and catheterization[2]. Patients with septicemia due to ESBL-producing organisms had a significantly higher fatality rate than those with non-ESBL-producing isolates[6]. A recent report from the Infectious Diseases Society of America listed ESBL-producing Klebsiella species and Escherichia coli as two of the six drug-resistant microbes to which new therapies are urgently needed[7]. Due to the increasing importance of multiresistant ESBL-producing Escherichia coli in the community, clinicians should be aware of the potential of treatment failures associated with serious infections caused by these bacteria[8].

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The emergence of ESBL-producing E. coli infections in nonhospitalized patients has been recently described in several countries[9–11].In neonates (0–28 days) ESBL-producing Klebsiella pneumoniae is an important cause of nosocomial infections[4]. However, limited information is available on these infections in children especially neonates. The present study is directed to determine the frequency of infections caused by ESBL-producing organisms, the various bacteria producing ESBL, and the antibiotic susceptibility of these organisms in a neonatal intensive care unit in a tertiary care hospital of Bihar.

Methodology

This study was conducted in the tertiary-level referral and inborn neonatal units by the Department of Microbiology at GMC, Bettiah, Bihar, India, from March 2020 February 2021. Written informed consent was obtained for each subject from the parents. The study was approved by the institute's Ethics Committee. All patients with suspected neonatal sepsis admitted during the study period were included in the study. Suspected neonatal sepsis was defined as the presence of two or more of the following: a) neonates with two or more risk factors[12, 13], b) neonates with clinical features suggestive of sepsis[14] and c) neonates with positive sepsis screen[15, 16] Neonates in whom consent was denied and neonates in whom bacterial culture grew a mixture of organisms were excluded from the study.Relevant clinical samples were obtained from the suspected cases of neonatal sepsis during the study period as directed by their clinical condition. The samples were subjected to standard microbiological methods to isolate and identify the organism, to find the antibiotic susceptibility patterns, and to detect the ESBL-producing organisms. Antimicrobial susceptibility testing of all isolates was performed by Kirby Bauer disk diffusion method. In this method, the inoculums were adjusted to the turbidity of a 0.5 McFarland standard and swabbed onto the surface of a Muller-Hinton agar plate. After putting the disks onto the inoculated plates, the plates were incubated at 37°C for 24 hours. Antibiotic potency of the discs was standardized against the reference strain. All susceptibility results were interpreted according to the CLSI (Clinical and Laboratory

Standards Institute). The following antimicrobial agents were used for susceptibility testing: ampicillin (A), amikacin (Ak), aztreonam (Ao), ceftazidime (Ca), ceftazidime-clavulanic acid (Cac), cefotaxime (Ce), ceftriaxone (Ci), ciprofloxacin (Cf), cotrimoxazole (Co), cefepime (Cpm), chloramphenicol (C), gentamicin (G), meropenem (Mr), nalidixic acid (Na), nitrofurantoin (Nf), netilmicin (Nt), norfloxacin (Nx), ofloxacin (Of), and piperacillin-tazobactam (Pt). For detection of ESBL production, modified double-disk test was performed as a screening test. ESBL production and susceptibility to antimicrobial agents were detected on the same plate. Susceptibility testing was performed as previously described. Disks containing ceftazidime alone and a combination of clavulanic acid and ceftazidime were placed in a distance of 25 mm (centre to centre). The zones of inhibition for ceftazidime alone and ceftazidime plus clavulanic acid were compared. An increase in zone diameter of 5 mm in the presence of clavulanic acid indicated the presence of ESBL in the test organisms. Automated identification system (Microscan Walkaway, from Siemens) was used for reconfirmation of ESBL production.

A total of 120 cases were enrolled in the study during the study period. Data was analyzed using Statistical Package for Social Sciences ver. 20.0 (IBM, Chicago).

Results

A total of 120 neonates with suspect sepsis were enrolled in the study during the study period. Culture-positive gram-negative organisms were found in 42 neonates. Of these mixture of organisms was found in 5 neonates and was excluded from the study. Hence, a total of 37 culture-positive neonates were included in the study cohort. The study cohort was further divided into two cohorts—6 neonates with ESBL-positive cultures and 31 neonates with ESBL-negative culture. Hence, the incidence of ESBL producing organism was 5%. The baseline characteristics of both ESBL-positive and -negative cohorts are illustrated in Table 1.

Table 1: Baseline characteristics of the study cohorts

Characteristics	ESBL producing organism	Non-ESBL producing organism
Median age in days at the onset of sepsis	2	4
Mean gestational age	29	38
Gender, male (n)	5	19
Birthweight, normal (n)	1	17
Preterm delivery (n)	5	11
Mode of delivery, normal vaginal delivery (n)	3	21
Meconium stained amniotic fluid (n)	2	11
Clinical signs of sepsis (n)	4	18
Positive sepsis screen (n)	2	16
Early onset sepsis (n)	5	18

Klebsiella was isolated from 4 out of 6 ESBL positive samples and another 2 yielded Escherichia coli. Among the 31 ESBL negative culture, majority were Acenitobacter (51.6%) followed by Klebsiella (29%) and then Enterobacter (12.9%). Remaining the other 2, one was Escherichia coli and one was Pseudomonas. Most common positive samples were endotracheal tube tip and aspirate for both the cohorts followed by blood specimens.

The antimicrobial resistance patterns of the ESBL-producing and non-ESBL-producing organisms are given in Figure 1. Analysis of the antimicrobial resistance patterns reveals that ESBL-producing organisms were more resistant to beta-lactam antibiotics compared to non-ESBL-producing organisms.

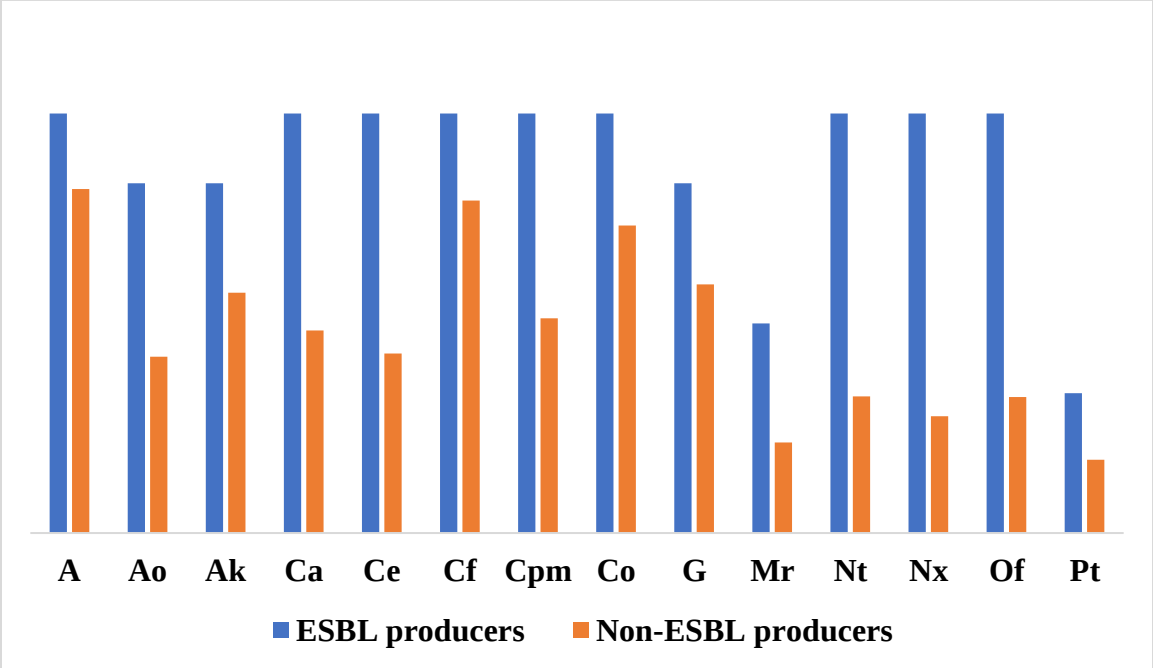

Figure 1: Antimicrobial pattern of ESBL and non-ESBL producing organism

Among the ESBL-positive cohort, five neonates improved and one expired. Among the ESBL-negative neonates, 26 neonates improved, two neonates left against medical advice, and three neonates expired.

Discussion

Infections caused by ESBL-producing organisms are a significant cause of neonatal morbidity and mortality all over the world mainly attributed to the widespread use of broad-spectrum antibiotics. The incidence of the infections caused by ESBL-producing organisms varies considerably in different geographical situations, from 37% in Latin America, 7% in the United States[17], to 5–56% in various Asian countries[5, 18–21]. In India, a recent study reported a 36.5% and 28.6% prevalence of ESBL-producing *E. coli* and *Klebsiella*, respectively, in neonatal infections[22]. Another similar study from India found the prevalence of ESBL-producing isolates of *E. coli* and *K. pneumonia* to be 22%[23]. The incidence of these infections in the present study is low as compared to the other studies in India

Klebsiella was the most common organism producing ESBL followed by *E. coli*. The bacterial spectrum in the present study is comparable to that in other studies[24–26]. Though a few studies have noted the ESBL-producing *Enterobacter* and *Acinetobacter* species, we did not find ESBL production in these organisms[26, 27]. These infections were mostly acquired in the perinatal or neonatal period from the hospital and therefore are multiresistant. Prior antibiotic use (ampicillins and cephalosporins) was found to be a significant risk factor for ESBL production which was specified as one of the major risk factors in other studies[28, 29].

The antimicrobial resistance patterns of both ESBL-producing and non-ESBL-producing organisms were comparable with those of other studies[26, 30]. Ampicillin, ciprofloxacin, and cotrimoxazole in general had higher resistance rates among both ESBL-producing and non-ESBL-producing organisms, with the reason being previous widespread use of these antibiotics. In a study, it was described that ESBL-producing *Klebsiella spp*. and ciprofloxacin resistance are closely associated[31]. Recently, the 2008 SMART (Study for Monitoring Antimicrobial Trends) results have emphasized on the alarmingly high rates of *E. coli* isolates resistant to fluoroquinolones in India[32]. The only hope for treating these infections lies in the carbapenems, piperacillin-tazobactam, and cefoperazone-sulbactam. Piperacillin-tazobactam was effective in majority of the ESBL producers in the present study which was in unison with a recent study from India[33]. Though many other resistance mechanisms for beta-lactam antibiotics like alteration of the penicillin binding proteins, low-affinity penicillin binding proteins, and alteration in the outer membrane permeability have been described in various gram-positive and gram negative cocci, ESBL production remains the main mechanism of resistance in gram-negative bacilli[34].

Conclusion

ESBL test should be routinely done in all culture-positive samples growing gram-negative organisms as the infections by ESBL-producing organisms are a significant problem in neonates. Judicious prescription of antibiotics is recommended as prior use of antibiotics is a significant risk factor for ESBL production. Strict aseptic precautions should be maintained in handling the neonates especially the preterm and low-birthweight neonates. Interventions and duration of hospital stay should be minimized as far as possible. Longitudinal surveillance of the microbial flora and their antibiotic sensitivity pattern should be done in every hospital periodically to know the existing flora and for appropriate management of the infection by these organisms.

References

1. U. Chaudhary and R. Aggarwal, "Extended spectrum β-lactamases (ESBL)—an emerging threat to clinical therapeutics," Indian Journal of Medical Microbiology, vol. 22, no. 2, pp. 75–80, 2004.
2. A. Ayyagari and A. Bhargava, "b-lactamases and their clinical significance (A mini review)," Hospital Today, vol. 6, no. 10, pp. 1–6, 2001.
3. S. Nathisuwan, D. S. Burgess, and J. S. Lewis II, "Extended-spectrum β-lactamases: epidemiology, detection, and treatment," Pharmacotherapy, vol. 21, no. 8, pp. 920–928, 2001.
4. J. Ena, F. Arjona, C. Martínez-Peinado, M. del mar López-Perezagua, and C. Amador, "Epidemiology of urinary tract infections caused by extended-spectrum beta-lactamase-producing Escherichia coli," Urology, vol. 68, no. 6, pp. 1169–1174, 2006.
5. A. Kaftandzhieva, V. Kotevska, G. Jankoska, B. Kjurcik-Trajkovska, Z. Cekovska, and M. Petrovska, "Extended-spectrum beta-lactamase-producing E. Coli and Klebsiella Pneumoniae in children at University Pediatric Clinic in Skopje," Macedonian Journal of Medical Sciences, vol. 2, no. 1, pp. 36–41, 2009.
6. B. Blomberg, R. Jureen, K. P. Manji et al., "High rate of fatal cases of pediatric septicemia caused by gram-negative bacteria with extended-spectrum beta-lactamases in Dar es Salaam, Tanzania," Journal of Clinical Microbiology, vol. 43, no. 2, pp. 745–749, 2005.
7. G. H. Talbot, J. Bradley, J. E. Edwards Jr., D. Gilbert, M. Scheid, and J. G. Bartlett, "Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America," Clinical Infectious Diseases, vol. 42, no. 5, pp. 657–668, 2006.
8. J. D. Pitout and K. B. Laupland, "Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern," The Lancet Infectious Diseases, vol. 8, no. 3, pp. 159–166, 2008.
9. F. W. Goldstein, "Antibiotic susceptibility of bacterial strains isolated from patients with community-acquired urinary tract infections in France," European Journal of Clinical Microbiology and Infectious Diseases, vol. 19, no. 2, pp. 112–117, 2000.
10. J. Rodríguez-Baño, M. D. Navarro, L. Romero et al., "Epidemiology and clinical features of infections caused by extended-spectrum beta-lactamase-producing Escherichia coli in nonhospitalized patients," Journal of Clinical Microbiology, vol. 42, no. 3, pp. 1089–1094, 2004.
11. R. Colodner, W. Rock, B. Chazan et al., "Risk factors for the development of extended-spectrum beta-lactamase-producing bacteria in nonhospitalized patients," European Journal of Clinical Microbiology and Infectious Diseases, vol. 23, no. 3, pp. 163–167, 2004.
12. M. Singh, A. Narang, and O. N. Bhakoo, "Predictive perinatal score in the diagnosis of neonatal sepsis," Journal of Tropical Pediatrics, vol. 40, no. 6, pp. 365–368, 1994.
13. V. P. Takkar, O. N. Bhakoo, and A. Narang, "Scoring system for the prediction of early neonatal infections," Indian Pediatrics, vol. 11, no. 9, pp. 597–600, 1974.
14. M. J. Sankar, R. Agarwal, A. K. Deorari, and V. K. Paul, "Sepsis in the newborn," Indian Journal of Pediatrics, vol. 75, no. 3, pp. 261–266, 2008.
15. C. Polinski, "The value of the white blood cell count and differential in the prediction of neonatal sepsis," Neonatal Network, vol. 15, no. 7, pp. 13–23, 1996.
16. O. Da Silva, A. Ohlsson, and C. Kenyon, "Accuracy of leukocyte indices and C-reactive protein for diagnosis of neonatal sepsis: a critical review," Pediatric Infectious Disease Journal, vol. 14, no. 5, pp. 362–366, 1995.
17. H. S. Sader, R. N. Jones, A. C. Gales et al., "Antimicrobial susceptibility patterns for pathogens isolated from patients in Latin American medical centers with a diagnosis of pneumonia: analysis of results from the SENTRY Antimicrobial Surveillance Program (1997)," Diagnostic Microbiology and Infectious Disease, vol. 32, no. 4, pp. 289–301, 1998.
18. J. M. Bell, J. D. Turnidge, A. C. Gales, M. A. Pfaller, and R. N. Jones, "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)," Diagnostic Microbiology and Infectious Disease, vol. 42, no. 3, pp. 193–198, 2002.
19. N. H. Ryoo, E.-C. Kim, S. G. Hong et al., "Dissemination of SHV-12 and CTX-M-type extended-spectrum β-lactamases among clinical isolates of Escherichia coli and Klebsiella pneumoniae and emergence of GES-3 in Korea," Journal of Antimicrobial Chemotherapy, vol. 56, no. 4, pp. 698–702, 2005.
20. X. Jiang, Y. Ni, Y. Jiang et al., "Outbreak of infection caused by Enterobacter cloacae producing the novel VEB-3 beta-lactamase in China," Journal of Clinical Microbiology, vol. 43, no. 2, pp. 826–831, 2005.
21. Y. Hirakata, J. Matsuda, Y. Miyazaki et al., "Regional variation in the prevalence of extended-spectrum β-lactamase-producing clinical isolates in the Asia-Pacific region (SENTRY 1998–2002)," Diagnostic Microbiology and Infectious Disease, vol. 52, no. 4, pp. 323–329, 2005.
22. S. Shakil, S. Z. Ali, M. Akram, S. M. Ali, and A. U. Khan, "Risk factors for extended-spectrum β-lactamase producing Escherichia coli and Klebsiella pneumoniae acquisition in a neonatal intensive care unit," Journal of Tropical Pediatrics, vol. 56, no. 2, pp. 90–96, 2009.
23. P. Agrawal, A. Ghosh, S. Kumar, B. Basu, and K. Kapila, "Prevalence of extended-spectrum β-lactamases among Escherichia coli and Klebsiella pneumoniae isolates in a tertiary care hospital," Indian Journal of Pathology and Microbiology, vol. 51, no. 1, pp. 139–142, 2008.
24. P. K. Das, K. Basu, P. Chakraborty, and P. K. Bhowmik, "Clinical and bacteriological profile of neonatal infections in metropolitan city based medical college nursery," Journal of the Indian Medical Association, vol. 97, no. 1, pp. 3–5, 1999.
25. H. Kapoor, M. Sumathi, P. Aggarwal, S. D. Jain, and J. Kaur, "Spectrum of bacterial isolates in high risk areas of a tertiary care hospital: 3 year study," Indian Journal of Medical Microbiology, vol. 18, pp. 166–169, 2000.
26. A. Jain, I. Roy, M. K. Gupta, M. Kumar, and S. K. Agarwal, "Prevalence of extended-spectrum β-lactamase-producing Gram-negative bacteria in septicaemic neonates in a tertiary care hospital," Journal of Medical Microbiology, vol. 52, no. 5, pp. 421–425, 2003.
27. Z. A. Bhutta, "Enterobacter sepsis in the newborn—a growing problem in Karachi," Journal of Hospital Infection, vol. 34, no. 3, pp. 211–216, 1996.
28. H. Abdel-Hady, S. Hawas, M. El-Daker, and R. El-Kady, "Extended-spectrum β-lactamase producing Klebsiella pneumoniae in neonatal intensive care unit," Journal of Perinatology, vol. 28, no. 10, pp. 685–690, 2008.
29. V. C. Cassettari, I. R. da Silveira, M. Dropa et al., "Risk factors for colonisation of newborn infants during an outbreak of extended-spectrum β-lactamase-producing Klebsiella pneumoniae in an intermediate-risk neonatal unit," Journal of Hospital Infection, vol. 71, no. 4, pp. 340–347, 2009.
30. A. Subha, S. Ananthan, and S. V. Alavandi, "Extended spectrum beta lactamase production & multidrug resistance in Klebsiella species isolated from children under five with intestinal & extraintestinal infections," Indian Journal of Medical Research, vol. 113, pp. 181–185, 2001.
31. D. L. Paterson, L. Mulazimoglu, J. M. Casellas et al., "Epidemiology of ciprofloxacin resistance and its relationship, to extended-spectrum β-lactamase production in Klebsiella pneumoniae isolates causing bacteremia," Clinical Infectious Diseases, vol. 30, no. 3, pp. 473–478, 2000.
32. P.-R. Hsueh, R. E. Badal, S. P. Hawser et al., "Epidemiology and antimicrobial susceptibility profiles of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections in the Asia-Pacific region: 2008 results from SMART (Study for Monitoring Antimicrobial Resistance Trends)," International Journal of Antimicrobial Agents, vol. 36, no. 5, pp. 408–414, 2010.
33. R. Sehgal, R. Gaind, H. Chellani, and P. Agarwal, "Extended-spectrum β lactamase-producing gram-negative bacteria: clinical profile and outcome in a neonatal intensive care unit," Annals of Tropical Paediatrics, vol. 27, no. 1, pp. 45–54, 2020.
34. A. Zapun, C. Contreras-Martel, and T. Vernet, "Penicillin-binding proteins and β-lactam resistance," FEMS Microbiology Reviews, vol. 32, no. 2, pp. 361–385, 2021.