

Prevalence & antimicrobial resistance pattern of extended spectrum β -lactamase producing *Klebsiella* spp isolated from cases of neonatal septicaemia

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Background & objectives: Extended spectrum β -lactamase (ESBL) producing *Klebsiella* spp led to serious concern about septicaemic neonates in neonatal intensive care units (NICU) due to high resistance against commonly used antimicrobial agents. Knowledge of disease burden and information on resistance to antimicrobials are required for proper management of such cases in NICUs. Here we report the prevalence and resistance pattern of ESBL producing *Klebsiella* spp isolated from cases of neonatal septicaemia at a tertiary care hospital from north India.

Methods: A total of 100 clinical isolates of *Klebsiella* spp isolated from 2995 blood samples of suspected cases of neonatal septicaemia were studied. Antimicrobial susceptibility was determined by Kirby- Bauer's disc diffusion method. All isolates were screened for ESBL production on the basis of inhibition zone against cephotaxime (<27 mm) and ceftazidime (<22 mm) and a breakpoint of minimum inhibitory concentration (MIC) (<2 μ g/ml for cephotaxime and <8 μ g/ml for cefpodoxime) by agar dilution method. Resistance pattern of ESBL producers and non-ESBL producers was compared.

Results: Of the 100 *Klebsiella* isolates, 58 were positive for ESBL production, which was much lower than 86.6 per cent reported in 2003. Almost all the isolates were sensitive to imipenam and meropenam. Drug resistance was found to be significantly more common in ESBL producing isolates than in non-ESBL producers.

Interpretation & conclusion: We found that 56 per cent of *Klebsiella* spp isolates were ESBL producers. There is a need to carefully formulate therapeutic strategies to control infections in NICUs. The high percentage of drug resistance in ESBL producing *Klebsiella* spp suggests that routine detection of ESBL is required by reliable laboratory methods.

Key words Extended spectrum β -lactamase (ESBL) - Gram-negative septicaemia - *Klebsiella* spp - neonatal septicaemia

Septicaemia is a leading cause of neonatal mortality and a significant proportion of newborns are infected with *Klebsiella* spp in NICUs^{1,2}. Extended spectrum β -lactamase (ESBL) producing *Klebsiella* spp have been frequently implicated in outbreaks in NICUs and account for a vast range of resistance against antimicrobial agents^{1,2}.

ESBL producing *Klebsiella* spp were first reported in 1983 from Germany³, and since then a steady increase of strains resistant to cephalosporins has been seen. The emergence of ESBL producing strains derived from mutation in TEM and SHV enzymes, which are present in 75 per cent of enterobacteriaceae isolates, is documented⁴. ESBLs are more prevalent in *Klebsiella* spp than any other enterobacterial species and outbreaks of infection caused by ESBL producing *Klebsiella* spp have been widely reported⁵⁻⁸. From India, the high prevalence of ESBL producing *Klebsiella* spp. is reported varying from 6 to 87.0 per cent^{9,10}. In our previous study in a tertiary care centre in north India¹, we found that 11.8 per cent of neonatal septicaemia was caused by *Klebsiella* spp, and of the total *Klebsiella* isolates, 86.6 per cent were ESBL producers. This study was undertaken to see if there was any change in the prevalence of ESBL producing *Klebsiella* spp. isolated from cases of neonatal septicaemia in the same centre. The resistance pattern of these isolates was also determined.

Material & Methods

A total of 2995 blood samples from the same number of suspected cases of neonatal septicaemia, sent to the Department of Microbiology, K.G. Medical University, Lucknow, India, for blood culture during a period of two years (January 2004 - December 2005) were included in the study. Briefly, 1- 2 ml of blood was collected into 10 ml of brain heart infusion broth with 0.05 per cent sodium polyanethol sulphonate. The broth was incubated at 37°C, overnight. A blind subculture on MacConkey

agar plate, chocolate agar and blood agar plate (Hi-media, Mumbai) was done after 18 h. If no growth was obtained, the bottles were examined daily for seven days. Any sign of growth was followed by subculture and identified by Gram staining. Gram-negative rods were identified by relevant biochemical test *i.e.*, motility test, Methyl Red-Voges-Proskauer test, and sugar fermentation test¹¹.

Antimicrobial susceptibility test: Antimicrobial susceptibility was determined by Kirby-Bauer's disc diffusion method as per National Committee for Clinical Laboratory Standards (NCCLS) recommendations^{12,13}. Antimicrobial discs (μ g) used were ampicillin (10), amoxicillin/clavulanic acid (10/20), piperacillin (100), piperacillin/tazobactam (100), ticarcillin (75), ticarcillin/clavulanic acid (75/10), cefixime (5), cefuroxime (30), cefpodoxime (10), cephodoxime (30), ceftazidime (30), aztreonam (30), netilmycin (30), amikacin (30), gentamycin (30), chlorophenicol (30), cotrimoxazol (30), tetracycline (30), imipenam (10) and meropenam (10). All these antibiotics were purchased from Hi-media Laboratories, Mumbai. Quality control was achieved by using standard strain of *Klebsiella* ATCC70063 (gifted by Christian Medical College, Vellore). Isolates showing inhibition zones <27 mm for cephodoxime and <22 mm ceftazidime was identified as potential ESBL producers and again tested on the basis of minimum inhibitory concentration (MIC) and confirmatory test.

MIC was determined by agar dilution methods for cephodoxime (0.25-128 μ g/ml) and cefpodoxime (0.25-128 μ g/ml) using series of dilution according to NCCLS-2003 guidelines¹³. Inoculated plates were incubated in ambient air at 35°C for 16-20 h. The MIC of each antimicrobial agent was defined as the lowest concentration that inhibited visible growth of the organism. A breakpoint of MIC, <2 μ g/ml for cephodoxime and <8 μ g/ml for cefpodoxime was identified as marker of ESBL production¹³. Quality

control was achieved by using standard strain of *Klebsiella* ATCC70063.

Confirmatory test for ESBL production: The combined disk method was used to confirm the presence of ESBL on all the isolates of *Klebsiella* spp by placing a disk (μg) of ceftazidime (30) alone and ceftazidime (30) in combination with clavulanic acid (10) on a Muller-Hinton agar plate. The discs were placed at least 20 mm apart from each other. Two parameters were taken as indicator of ESBL production^{13,14}. (i) The zone diameter around ceftazidime + clavulanic acid disc is >5 mm larger than that around ceftazidime disc, confirms the presence of ESBL¹⁵. (ii) If ratio of zone diameter around discs with ceftazidime + clavulanic acid and ceftazidime alone is >1.5 , it confirms ESBL production¹⁴.

Quality control for ESBL detection: *K. pneumoniae* ATCC700603 (ESBL positive) was used as quality control for ESBL test. On disk diffusion testing the zone diameter (mm) ranges for *K. pneumoniae* ATCC700603 were as follows; cefpodoxime 9-16 mm, ceftazidime 10-18, aztreonam 9-17 and cephotoxime 17-25. In disc diffusion phenotypic testing, *K. pneumoniae* ATCC700603 shows >5 mm increase in ceftazidime/ clavulanic acid zone diameter¹³.

Statistical analysis: Chi-square test was used with appropriate correction for the observation. Where the cell frequency was less than five, Fisher exact tests was applied to see the significance between the resistance level of various drugs in ESBL producer and non-ESBL producer *Klebsiella* spp using STATA 8.2 software. $P \leq 0.05$ was considered significant.

Results & Discussion

A total of 100 isolates of *Klebsiella* spp. were isolated from 2995 blood samples (3.3%). Of these

100 isolates, 58 (58.0%) were ESBL positive. The antimicrobial resistance was significantly ($P < 0.05$) higher in ESBL producers than in non-ESBL producers. All the isolates were sensitive to imipenam and meropenam (except one). ESBL producing *Klebsiella* spp. were almost always resistant to ampicillin, ticarcillin and piperacillin. Monobactam and cephalosporin resistance was also higher in ESBL producing *Klebsiella* spp. Aminoglycosides *i.e.*, amikacin and gentamycin accounted for 58.6 and 70.6 per cent resistance among ESBL producers. Piperacillin/tazobactem showed less resistance as compared to ticarcillin/clavulanic acid and amoxicillin/clavulanic acid (Table).

Table. Comparison of antimicrobial resistance patterns of ESBL producer (n=58) and non-ESBL producer *Klebsiella* spp (n=42)

Drugs	ESBL producer (n=58)	Non ESBL producer (n=42)
Ampicillin	57	21 **
Ticarcillin	57	13 **
Piperacillin	57	14 **
Amox/Clavulanic acid	25	7
Ticarcillin/Clavulanic acid	43	4 **
Piperacillin/Tazobactem	15	10
Aztreonam	49	11 **
Imipenam	0	0
Meropenam	1	0 **
Ceftazidime	47	8 **
Cephotoxime	52	8 **
Ceufuroxime	48	2 **
Cefixime	53	5 **
Cefpodoxime	54	6 **
Amikacin	34	8 **
Gentamicin	41	22
Netilmicin	51	32
Tetracycline	55	34 *
Ciprofloxacin	39	8 **
Chloramphenicol	48	16 *
Cotrimoxazole	40	26

$P < 0.05$, ** < 0.001 compared to ESBL producers

The incidence of neonatal septicaemia caused by *Klebsiella* spp. as seen in the current study (3.3%) was much less in comparison of what we reported in 2003 (11.8%) from same NICU¹. There was also a remarkable decrease in prevalence of ESBL producing *Klebsiella* species as seen in isolates from the cases of neonatal septicaemia (86.6 vs. 58%).

In recent years a significant increase in ESBL producing *Klebsiella* spp. was reported from USA 4.2-44.0 per cent¹⁵⁻¹⁷, Canada, 4.9 per cent¹⁸ and China 51 per cent¹⁹. Focussing on the epidemiology in Europe, there are considerable geographical differences in the occurrence of ESBLs. A recent large survey of 1610 *Escherichia coli* and 785 *K. pneumoniae* isolates from 31 centers in 10 European countries found that the prevalence of ESBL in these organisms ranged from as low as 1.5 per cent in Germany to as high as 39-47 per cent in Russia, Poland and Turkey²⁰.

In India, high prevalence of ESBL producing *Klebsiella* strains has been reported by various groups. Reported frequency of ESBL producing *Klebsiella* spp. from India ranged between 6 and 87 per cent^{9,10}. Prevalence of ESBL producing *Klebsiella* spp. as reported by other investigators was 25.6, 25.8, 30.18, 80.0²¹⁻²⁴ and 86.6 per cent¹.

The high percentage of ESBL producing *Klebsiella* spp may be due to the selective pressure imposed by extensive use of antimicrobials. Intensive care unit, in which antibiotic use is heaviest and the potential for patient-to-patient transmission of organisms is greatest, is an important factor. The infection control implications of ESBL producing *Klebsiella* spp. are under-recognized. In most of the studies, molecular genetic evidences indicated patient-to-patient transmission of ESBL producing strains of *Klebsiella* spp. More than 50 hospital outbreaks of infection with ESBL producing *Klebsiella* have now been reported²⁵.

In the present study resistance to three or more drugs (multi drug resistance, MDR) was common in ESBL producers than non-ESBL producers. About 95.0 per cent ESBL producers were resistant to penicillins and more than 85.0 per cent to cephalosporins. However, NCCLS documents¹³ recommend that ESBL producers should not be reported as susceptible to cephalosporins, since the ESBLs destroy these drugs and ESBL producing bacteria will remain resistant to treatment with these drugs. Carbapenems are considered the last resort in NICU for ESBL producing isolates and resistance to carbapenems is a serious concern and has been reported in certain hospitals²⁶⁻²⁸. In our study, only one isolate showed the resistance against meropenam. The resistance may be due to reduced levels of drug accumulation or increased expression of pump efflux or may be due to the production of metallo- β -lactamase as seen in *Pseudomonas* spp²⁹.

In conclusion, our results showed a decrease in ESBL producing *Klebsiella* spp. in our NICU. The immense use of broad spectrum cephalosporins has become one of the major factors responsible for the high rate of selection of ESBL producing microorganisms. Routine detection of ESBL producing microorganisms is required by reliable laboratory methods and since most of these are multidrug resistant, the therapeutic strategies to control infections in NICUs has to be carefully formulated.

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References

1. Jain A, Roy I, Gupta MK, Kumar M, Agarwal SK. Prevalence of extended spectrum beta-lactamase producing Gram-negative bacteria in septicaemic neonates in a tertiary care hospital. *J Med Microbiol* 2003; 52 : 421-5.

2. Boo NY, Ng SF, Lim VK. A case control study of risk factors associated with rectal colonization of extended spectrum beta-lactamase producing *Klebsiella* spp. in newborn infants. *J Hosp Infect* 2005; 61 : 68-74.
3. Knothe H, Shah P, Krcmery V, Antal M, Mitsuhashi S. Transferable resistance to cephotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. *Infection* 1983; 11 : 315-7.
4. Livermore DM. β -lactamase in laboratory and clinical resistance. *Clin Microbiol Rev* 1995; 8 : 557-84.
5. Hanberger H, Garcia Rodriguez JA, Gobernado M, Goosens H, Nilsson LE, Strulens MJ. Antibiotic susceptibility among aerobic gram-negative bacilli in intensive care units in 5 european countries, French and Portuguese ICU study groups. *JAMA* 1999; 281 : 67-71.
6. Albertini MT, Benoit C, Berardi L, Berrouane Y, Boisivon A, Cahen P, *et al.* Surveillance of methicillin resistant *Staphylococcus aureus* (MRSA) and enterobacteriaceae producing extended spectrum beta-lactamase (ESBLE) in Northern France: a five years multicentre incidence study. *J Hosp Infect* 2002; 52 : 107-13.
7. Desimoni MC, Esquivel GP, Merino LA. Fecal colonization by extended spectrum beta-lactamase producing *Klebsiella pneumoniae* in a neonatal intensive care unit. *Enferm Infecc Microbiol Clin* 2004; 22 : 507-11.
8. Duman M, Abacioglu H, Karaman M, Duman N, Ozkan H. Beta-lactam antibiotic resistance in aerobic commensal fecal flora of newborns. *Pediatr Int* 2005; 47 : 267-73.
9. Hansotia JB, Agarwal V, Pathak AA, Saoji AM. Extended spectrum beta-lactamase mediate resistance to third generation cephalosporins in *Klebsiella pneumoniae* in Nagpur, central India. *Indian J Med Res* 1997; 105 : 160-5.
10. Manchanda V, Singh NP, Goyal R, Kumar A, Thukral SS. Phenotypic characteristics of clinical isolates of *Klebsiella pneumoniae* and evaluation of available techniques for detection of extended spectrum beta-lactamases. *Indian J Med Res* 2005; 122 : 330-7.
11. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Tenover FC, editors. The enterobacteriaceae. In: *Color atlas & textbook of diagnostic microbiology*, 5th ed. Philadelphia: JB Lippincott Co.; 1997 p.171-230.
12. Bauer AW, Kirby WMM, Sherris JC, Tuck M. Antibiotics susceptibility testing by standardized single disc method. *Am J Clin Pathol* 1966; 45 : 493-6.
13. National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial susceptibility testing*. 13th Informational Supplement. Wayne, Pennsylvania: National Committee for Clinical Laboratory Standards Document M100-S13; 2003.
14. M'Zali FH, Chanawong A, Kerr KG, Birkenhead D, Hawkey PM. Detection of extended spectrum beta-lactamases in members of the family enterobacteriaceae: comparison of the MAST DD test, the double disc and the E test ESBL. *J Antimicrob Chemother* 2000; 45 : 881-5.
15. Mathai D, Lewis MT, Kugler KC, Pfaller MA, Jones RN. SENTRY Participant Group (North America). Antibacterials activity of 41 antimicrobials tested against over 2773 bacterial isolates from hospitalized patients with pneumonia: I-results from the SENTRY antimicrobial surveillance program (North America, 1998). *Diagn Microbiol Infect Dis* 2001; 32 : 105-16.
16. Saurina G, Quale GM, Manikal VM, Oydna E, Landman D. Antimicrobial resistance in enterobacteriaceae in Brooklyn NY: Epidemiology and relation to antibiotic usage patterns. *J Antimicrob Chemother* 2000; 45 : 895-8.
17. Winokur PL, Canton R, Caselass JM, Legakis N. Variations in the prevalence of strains expressing an extended spectrum beta-lactamase phenotype and characterization of isolates from Europe, the Americas, and the western pacific region. *Clin Infect Dis* 2001; 32 (Suppl.2): S94-103.
18. Cordero L, Rau R, Taylor D, Avers LW. Enteric gram-negative bacilli bloodstream infections: 17 year's experience in a neonatal intensive care unit. *Am J Infect Control* 2004; 32 :189-95.
19. Xiong Z, Zzhu D, Zhang Y, Wang F. Extended spectrum beta-lactamase in *Klebsiella pneumoniae* and *Escherichia coli* isolates. *Zhonghua Yi Xue Za Zhi* 2002; 82 : 1476-9.
20. Goosens H. MYSTIC programme: summary of European data from 1997 to 2000. *Diagn Microbiol Infect Dis* 2001; 41 : 183-9.
21. Tankhiwale SS, Jalgaonkar VS, Ahamad S, Hassani U. Extended spectrum beta-lactamase in urinary isolates. *Indian J Med Res* 2004; 120 : 553-6.

22. Shubha S, Ananthan S. Extended spectrum beta-lactamase (ESBL) mediated resistance to third generation cephalosporins among *Klebsiella pneumoniae* in Chennai. *Indian J Med Microbiol* 2002; 20 : 92-5.
23. Shukla I. Prevalence of extended spectrum beta-lactamase in *Klebsiella pneumoniae* in a tertiary care hospital. *Indian J Med Microbiol* 2004; 22 : 87-91.
24. Mathur P, Kapil A, Das B, Dhawan B. Prevalence of extended spectrum beta-lactamase producing Gram negative bacteria in a tertiary care hospital. *Indian J Med Res* 2005; 122 : 305-8.
25. Patterson DL, Ko W-C, Gotberg AV, Mohapatra S, Cassels JM, Goosness H, *et al.* International prospective study of *Klebsiella pneumoniae* bacteremia: implications of extended spectrum beta-lactamase production in nosocomial infections. *Ann Intern Med* 2004; 140 : 26-32.
26. Gupta E, Mohanty S, Sood S, Dhawan B, Das B, Kapil A. Emerging resistance to carbapenam in a tertiary care hospital in north India. *Indian J Med Res* 2006; 124 : 95-8.
27. Kurokawa H, Yagi T, Shibata N, Arakawa Y. Worldwide proliferation of carbapenam-resistant gram-negative bacteria. *Lancet* 1999; 354 : 955.
28. Yano H, Kuga A, Okamoto R, Kitasato H, Koyabashi T, Inone M. Plasmid coded metallo beta-lactamase (imp 6) conferring resistance to carbapenams, especially meropenams. *Antimicrob Agents Chemother* 2001; 45 : 1343-8.
29. Navneeth BV, Sridaran D, Sahay D, Belwadi MRS. A preliminary study on metallo β -lactamase producing *Pseudomonas aeruginosa* in hospitalized patients. *Indian J Med Res* 2002; 116 : 264-7.

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