

Original Article

A STUDY OF EXTENDED SPECTRUM β -LACTAMASE (ESBL) AND AmpC β -LACTAMASE PRODUCING KLEBSIELLA PNEUMONIAE IN NEONATAL INTENSIVE CARE UNIT AT TERTIARY CARE HOSPITAL, AHMEDABAD

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ABSTRACT

Background & objectives: Clinical laboratories need to develop quick screening methods for detection of Extended Spectrum β -Lactamases (ESBL) & Amplified C (AmpC) β -Lactamase, so that the appropriate medication can be started without delay. Here, we reported the screening & confirmatory methods for detection of ESBL & AmpC in Klebsiella pneumoniae in Neonatal Intensive Care Unit (NICU).

Methods: We had tested 600 blood culture samples from the NICU patients. From the positive bacterial isolates, Klebsiella pneumoniae were screened for ESBL & AmpC production followed by confirmatory methods as per Clinical Laboratory Standard Institute (CLSI) guidelines.

Results: Out of the 600 blood culture, 266 were positive for microbial growth. Among them, Klebsiella pneumoniae were in 54 patients. Out of 54 Klebsiella pneumoniae, 48 were positive for ESBL in screening method. Out of 48, 41 isolates were ESBL positive & 4 were AmpC positive by Confirmatory test (Modified Three Dimensional Method for AmpC).

Interpretation & conclusions: The prevalence of ESBL & AmpC producing Klebsiella pneumoniae in NICU at our institute is 75.92% & 7.4% which is very alarming, and it requires strict implementation of infection control guidelines in NICU by safe hygiene practices, restricted use of broad spectrum antibiotics as empirical therapy in septicemic cases and also formulation of uniform antibiotic policy for such patients based on the current trend of antibiotic resistance. This can be helpful in preventing emergence of multidrug resistance in such organisms.

Key words: ESBL, AmpC, *Klebsiella pneumoniae*, Neonatal septicemia.

INTRODUCTION

Septicaemia is one of the leading causes of neonatal mortality along with perinatal hypoxia. The most common organisms responsible for these infections are multidrug resistant gram negative bacilli particularly members of the family *Enterobacteriaceae*.¹ Several outbreaks of septicaemia by gram negative isolates have been reported and phenomenon of isolation of ESBLs producing isolates is not uncommon and is associated with increased mortality.¹

Extended spectrum B- lactamases (ESBLs) are plasmid mediated, TEM and SHV derived enzymes, first isolated in Western Europe in mid 1980s, most commonly in *Klebsiella* spp., followed by *Escherichia coli*. These enzymes are capable of hydrolyzing broad spectrum cephalosporins, penicillins and monobactams such as aztreonam, but inactive against cephamycins and imipenem & are usually inhibited by β -Lactamase inhibitors such as clavulanic acid. In addition, Plasmids responsible for ESBL production tend to be large (80 Kb or more in size) and carry resistance to several agents, an important limitation in the design of treatment alternatives. The most frequent co-resistances found in ESBL producing organisms are aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and sulfamethoxazole-trimethoprim resulting in limitation of therapeutic option.² Furthermore, due to the variable affinity of these enzymes for different substrates and inoculum effect, some ESBL isolates may appear susceptible to a third generation cephalosporin in vitro. However, treatment of infections due to an ESBL producing organism with third generation cephalosporins may result in clinical failure.²

AmpC β - lactamases are cephalosporinases that are poorly inhibited by clavulanic acid & can be differentiated from ESBLs by their ability to hydrolyse cephamycins. Now a day, *Klebsiella pneumoniae* is also found to produce AmpC β -Lactamases. The increased prevalence of bacterial pathogens producing both ESBLs & AmpC β -Lactamases creates requirement for the laboratory testing methods that can accurately detect the presence of these enzymes in clinical laboratories.³

The study was conducted to know prevalence of ESBL & AmpC production in *Klebsiella pneumoniae* in NICU. We also studied antibiotic sensitivity pattern of ESBL producing isolates of

Klebsiella pneumoniae so that we can help clinicians to avoid injudicious use of antibiotics and giving susceptible antimicrobials to prevent therapeutic failure in neonates having infection with ESBL positive *Klebsiella pneumoniae*.

MATERIALS & METHODS

The present study was carried out from February 2009 to July 2009 in Microbiology Department of Tertiary Care Medical Centre affiliated with Medical College in Western India. A total of 600 samples of blood culture from neonates admitted in the NICU were processed for the study.

After taking universal precautions, blood for the culture was collected aseptically. Two ml blood was collected in brain heart infusion broth prepared for paediatric use. Blood culture bottles were sent immediately to the Microbiology laboratory.^{4,5} In the laboratory, blood culture samples were processed by the standard methods. *Klebsiella pneumoniae* were stamped by their colony morphology & standard biochemical reactions.⁵

As per the CLSI (Clinical Laboratory Standards Institute) guidelines, antibiotic susceptibility testing was performed by Kirby-Bauer method. Quality Control strains, *E.coli* ATCC 25922 (β -Lactamase negative) and *Klebsiella pneumoniae* ATCC 700603 (ESBL positive) were used.⁶

Screening test for ESBL detection:

Screening for ESBL was done according to the CLSI guidelines. All *Klebsiella pneumoniae* isolates were screened for ESBL production by Kirby-Bauer's disc diffusion method, demonstrating reduced susceptibility to cefpodoxime (30 μ g), cefotaxime (30 μ g), ceftazidime (30 μ g), ceftriaxone(30 μ g) and aztreonam (30 μ g). Cut-off zone sizes as an indicator of ESBL producer were \leq 27 mm for cefotaxime, \leq 22 mm for ceftazidime, \leq 25 mm for ceftriaxone, \leq 27 mm for aztreonam and \leq 17 mm for cefpodoxime.⁶

Confirmatory Test for ESBL Detection: Phenotypic Confirmatory Disc Diffusion Test (PCDDT):

This was done by placing a disk of ceftazidime (30 μ g) alone and ceftazidime + clavulanic acid (30/10 μ g) on a Mueller-Hinton Agar plate at

least 20 mm apart from each other. After overnight incubation plates were examined. The zone diameter around ceftazidime + clavulanic acid disc ≥ 5 mm larger than that around ceftazidime disk was indicated as ESBL production.⁶

Screening Test for AmpC β -lactamases:

An isolate was screened for AmpC β -Lactamases by Kirby-Bauer's disc diffusion method demonstrating reduced susceptibility to cefoxitin (30 μ g) as ≤ 18 mm zone of inhibition.⁷

Confirmatory Test for AmpC β -lactamase: Modified Three-Dimensional Test (MTDT):

The isolates showing positive screening test result by cefoxitin disc diffusion test was confirmed by MTDT. Briefly, fresh overnight growth from Mueller Hinton Agar was transferred to a pre-weighed sterile micro-centrifuge tube. The tube was weighed again to determine the weight of bacterial mass to obtain 10-15 mg of bacterial wet weight. The bacterial mass was suspended in peptone water and pelleted by centrifugation at 3000 rpm for 15 minutes. Crude enzyme extract was prepared by freezing and thawing the bacterial pellet (five cycles).

Lawn culture of *E. coli* ATCC 25922 was prepared on Mueller Hinton Agar plates, and a cefoxitin (30 μ g) disc was placed on the surface of the medium. Linear slits (3 cm long) were cut using sterile surgical blade up to a point 3 mm away from the edge of the cefoxitin disc. Wells of 8 mm diameter were made on the slits at a distance 5 mm inside from the outer end of the slit using a sterile pasteur pipette. The wells were loaded with enzyme extract in 10 μ l increments until the well was full. Approximately 30-40 μ l of extract was loaded in a well.

The plates were incubated at 37 °C for overnight. Three different kinds of results were recorded. Isolates that showed clear distortion of zone of inhibition of cefoxitin were taken as AmpC producers. Isolates with no distortion were taken as AmpC non-producers.^{7,8}

RESULTS & OBSERVATIONS

In this study, total of 600 blood culture samples from NICU patients suspected of having

septicemia were processed for the detection of *Klebsiella pneumoniae* with ESBL & AmpC production.

Out of 600 cases, growth was obtained in 266 patients. Among them, 256 were positive for bacteria & 10 for *candida spp.*(figure-1)

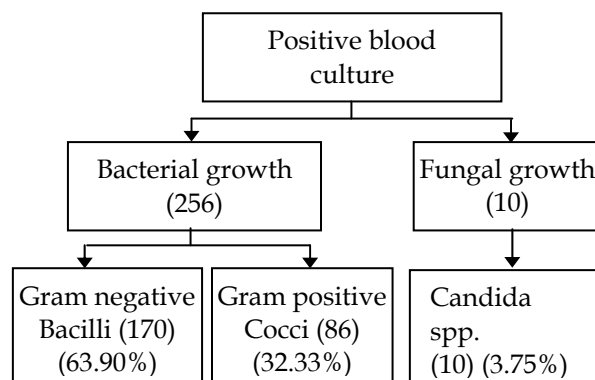


Figure:1 Incidence of Neonatal septicemia in neonatal intensive care unit

Klebsiella spp. were isolated in 63 isolates out of total 256 bacterial isolates.(Table-1) Out of total 63 *Klebsiella species*, *Klebsiella pneumoniae* were 54, while *Klebsiella oxytoca* were 6 & *Klebsiella ornitholytica* were 3 in number.

Table 1: Bacteriological profile of neonatal septicemia

Organism isolated from neonatal septicemia	Positive isolates(%)
Staphylococcus coagulase negative	73 (27.44)
<i>Klebsiella species</i>	63 (23.68)
<i>Pseudomonas aeruginosa</i>	42 (15.78)
<i>Acinetobacter species</i>	37 (13.90)
<i>E. coli</i>	26 (9.77)
<i>Candida species</i>	10 (3.75)
<i>Staphylococcus aureus</i>	08 (3.00)
<i>Streptococcus species</i>	03 (1.12)
<i>Proteus vulgaris</i>	01 (0.37)
<i>Morganella species</i>	01 (0.37)
<i>Enterococcus species</i>	01 (0.37)
Total	266 (100)

In screening test, out of 54 *Klebsiella pneumoniae*, 48 the *Klebsiella pneumoniae species* had exhibited resistance to at least any one of the five indicator antibiotics (cefotaxime, ceftazidime, cefpodoxime, ceftriaxone & aztreonam) & 4 had exhibited resistance to cefoxitin. So, 48 isolates

were positive for ESBL & 4 isolates were positive for AmpC enzyme by screening test.

Table:2 Antibiotic resistance pattern of ESBL producing *Klebsiella pneumoniae*.

Name of antibiotic	Susceptible isolates	% of Resistance
Cefaclor	41	100
Ceftazidime	41	100
Cefotaxime	41	100
Ceftriaxone	41	100
Cefpodoxime	41	100
Cefepime	38	92.68
Aztreonam	41	100
Cefoxitin	00	00
Amoxicillin-clavulanic acid	00	00
Piperacillin/tazobactam	09	21.95
Imipenem	00	00
Moxifloxacin	02	4.87
Amikacin	12	29.26
Cotrimoxazole	32	78.04
Tetracycline	39	95.12



Figure 2: Positive phenotypic confirmatory disk diffusion test by CLSI for ESBL showing ≥ 5 mm increase in zone diameter of ceftazidime (CA) in the presence of inhibitor clavulanic acid (CAC), synergism is also seen between these two antibiotics.

Out of these 48, ESBL production was detected in 41 isolates by Phenotypic confirmatory disk diffusion method. So the prevalence of ESBL producing *Klebsiella pneumoniae* in our Neonatal ICU was 75.92% (41/54).

The AmpC production in all 4 isolates was confirmed by modified three dimensional test for AmpC enzyme. Thus the prevalence of AmpC enzyme in *Klebsiella pneumoniae* is 7.40% (4/54).

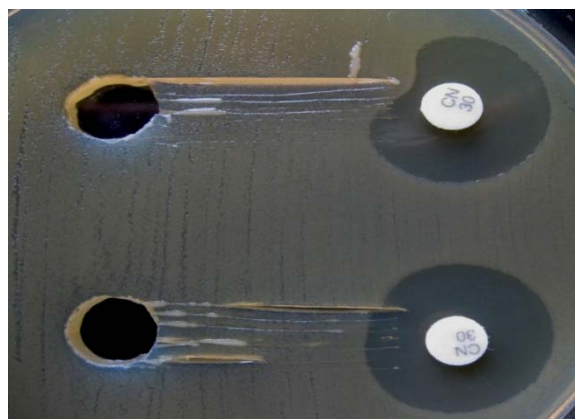


Figure 3: Modified three dimensional test for AmpC; positive test (above) showing distortion of zone of inhibition around cefoxitin (CN) disc & negative test (below) showing clear zone of inhibition around cefoxitin (CN) disc

DISCUSSION

Neonatal septicaemia is one of the most important causes of neonatal mortality. So, timely detection of septicaemia in neonates & initiation of proper antibiotic treatment can help to save the life.

In our study, the incidence of neonatal septicaemia confirmed by blood culture was 44.33% (266/600). The studies done on various parts of the India had reported the same findings. It was 48.14%¹, 52.36%⁹ & 47.5%¹⁰ in the studies done by A Bhattacharjee et al at S. S. Hospital, Banaras, Hindu university, Varansi in year 2008; DS Murty et al at Gandhi Hospital, Secunderabad in 2002 & I Roy et al at King George's Medical college, Lucknow in 2002 respectively. Although the common factors associated with neonatal infections are low birth weight, length of time spent in hospital, invasive procedures, surgery and also colonisation by bacteria from hospital environment, a significant proportion of these septicemic babies are those, who were born unattended outside the hospital in unhygienic environment and then referred to the tertiary care center for life threatening complications.¹

Prevalence of *Klebsiella pneumoniae* in NICU in our study was reported as 54/266 isolates (20.30%). DS Murty & I Roy had reported the prevalence of *Klebsiella pneumoniae* in NICU as 26.92% & 24.6% which was slightly higher than our study.^{9,10} While lower rate of prevalence was seen in the studies done by A bhattacharjee & Eddy Vercauteren as 13.67% & 15.1%

respectively.^{1,11} But in most of the studies *Klebsiella pneumoniae* was seen as predominating bacterial isolate responsible for fatal septicaemia in neonates.

As report of blood culture isolation and antibiotic susceptibility results are usually available after 72 hours or more, any delay in the initiation of correct empirical therapy or improper choice of antimicrobials cannot be justified. It is a common practice to use ampicillin and an aminoglycoside or a third generation cephalosporin in neonatal septicaemic cases.¹ Wide spread use of third generation cephalosporins and aztreonam is believed to be the major cause of the mutations in TEM and SHV enzymes that has led to the emergence of the ESBLs.

ESBL screening method recommended by CLSI for *E.coli* & *Klebsiella* species had identified that 48 isolates of *Klebsiella pneumoniae* out of 54 showed decreased susceptibility to at least any one indicator for ESBL detection. Thus in our study 88.88% *Klebsiella pneumoniae* were screen positive for ESBL detection test by CLSI criteria, while one study done by A. Bhattacharjee at Varanasi had recorded that 100%¹ *Klebsiella pneumoniae* were screened positive for ESBL detection test. ESBL production was confirmed by Phenotypic confirmatory disk diffusion method recommended by CLSI in 41 isolates. So the prevalence of ESBL producing *Klebsiella pneumoniae* in our Neonatal ICU was 75.92%. Brendan et al, Amita Jain et al, Bhattacharjee et al & I Roy et al had shown the prevalence of ESBL producing *Klebsiella pneumoniae* as 25%, 58%, 62.50% & 86.60% respectively.^{13,12,1,10} Major risk factors for colonization or infection with ESBL producing organisms are long term antibiotic exposure, prolonged ICU stay, nursing home residency, severe illness, residence in an institution with high rates of third generation cephalosporin use and instrumentation or catheterisation.² Our institute is the tertiary care medical center, so we have patients mainly referred from PHCs, CHCs and in addition also from private sectors due to complicated life threatening infections. This leads to use of higher antibiotics by the clinicians as a common empirical therapy without waiting for the susceptibility results. In addition, due to heavy patients load, proper infection control measures can not be maintained by the paramedical staff. Majority of the time, Intensive care units are occupied with the patients. So, proper fumigation of the ICUs

at regular interval is not possible. Also, the neonates having low birth weight or deficient immune response are more prone to the infections by the nursing staff or other medical staff if proper hygiene is not maintained while handling them in NICUs. In such cases, neonates are suffering from nosocomial infections caused by multidrug resistant organisms which are easily spreading in nature. All of these factors lead to the higher rate of ESBL and AmpC β -Lactamase producing organisms in our institute.

Antibiotic susceptibility pattern of ESBL positive isolates showed that all 2nd generation cephalosporins, 3rd generation cephalosporins and aztreonam were resistant. The cefepime was resistant in three isolates. The imipenem was sensitive in all ESBL producing isolates. Amoxiclav was sensitive in 41 cases while Piperacillin- tazobactam was sensitive in 32 cases. Co-resistance to other group of drugs was noted in ESBL producing organisms. In addition to imipenem, good activity against ESBL producing *Klebsiella pneumoniae* was noted by amikacin & moxifloxacin. Amikacin was sensitive in 29 cases while moxifloxacin was sensitive in 39 cases. So depending on the sensitivity pattern, these drugs can be given to the serious neonatal septicemic patient. Amikacin and higher quinolones are good alternatives and they will also provide some economic relief to the patients. Imipenem drug should be reserved for the future resource. Continued monitoring of the susceptibility pattern of the organisms is necessary in clinical settings to detect true burden of the antibiotic resistance for proper disease management.

CONCLUSION

Our institute which is tertiary care center of Gujarat (India) has a high prevalence of nosocomial infections in NICU due to *Klebsiella pneumoniae*. Thus incidence of Neonatal septicaemia in our study is 44.33% (266/600). The prevalence of *Klebsiella pneumoniae* in NICU patients is 20.30% (54/266). The prevalence of ESBL & AmpC producing *Klebsiella pneumoniae* in NICU at our institute is 75.92% & 7.4%. The higher prevalence rate of ESBL and AmpC producing organisms shows that in the future, problems in treatment of serious infections by MDR (Multi Drug Resistant) organism will be increasing. This result warns us to prevent intra - institutional spread of these organisms by

limiting the use of these agents alone or in combination with infection control measures. Educational programs for medical staff to increase awareness should also be developed. Antibiotic policy should be formulated for empirical therapy in high risk intensive care units where infections due to resistant organisms are much higher.

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