Middle East Research Journal of Microbiology and Biotechnology
ISSN: **2789-8644 (Print & Open Access)**
Frequency: Bi-Monthly
DOI: 10.36348/merjmb.2021.v01i01.005

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A Clinico-Microbiological Study of B-Lactamase Producing *Klebsiella Pneumoniae* Among Neonates From Neonatal Intensive Care Unit At Tertiary Care Hospital, Ujjain

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ABSTRACT: Infection caused by drug resistance *Klebsiella pneumoniae* is very common now days in community as well as nosocomial environment. *K.pneumoniae* is second most common pathogen in neonatal septicemia. Our aim to detect drug resistance *K.pneumoniae* by production of β-lactamase enzymes such as extended spectrum β-lactamase (ESBL), AmpC β-lactamase, metalo β-lactamase (MBL) and carbapenemase in neonatal septicemia. A total 13 isolates of *K.pneumoniae* detected from neonates admitted in tertiary care centre during study period from February 2015 to July 2016. We analyzed risk factors in K. *pneumoniae* infection among neonates on the basis of prolong rupture of membrane, preterm birth, onset of septicemia etc. Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion methods. β-lactamase enzymes includes, Extended spectrum β-lactamase(ESBL), AmpC β-lactamase, metalo β-lactamase (MBL)and carbapenemase were detected by phenotypic methods using standard guidelines. Distribution of MDR, XDR and PDR detected according to antimicrobial resistance pattern as per guideline. Among 13 *K.pneumoniae* isolate, antimicrobial resistance profile was studied. Isolates were 100% resistant to third generation cephalosporin and least resistant to imipenem 54%. MDR K.pneumoniae were 23 %, XDR were 77% and no PDR isolated. ESBL production seen in 23.1%, AmpC in 7.7 %, MBL in 00 % and Carbapenemase in 46.1 %. The study indicates that drug resistance higher in NICU of our institute. It requires strict implementation of infection control guidelines in NICU by safe hygiene practices, restricted use of broad spectrum antibiotics as empirical therapy and also formulation of uniform antibiotic policy for such patients based on the current trend of antibiotic resistance.

Keywords: K. pneumoniae, ESBL, Amp C, MBL, Carbapenemase, neonatal septicemia.

RESEARCH PAPER

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How to cite this paper:
Sonali Waske & Yogyata Marothi; "A Clinico-Microbiological Study of B-Lactamase Producing *Klebsiella Pneumoniae* Among Neonates From Neonatal Intensive Care Unit At Tertiary Care Hospital, Ujjain" Middle East Res J. Microbiol Biotechnol., 2021 Nov-Dec 1(1): 18-24.

Article History:
| Submit: 30.10.2021 |
| Accepted: 05.12.2021 |
| Published: 28.12.2021 |

INTRODUCTION

Neonatal septicemia is common cause of morbidity and mortality in neonatal intensive-care unit (NICU). It is more common in developing countries than developed due to poor practice of hand hygiene and infection control measures [1]. Incidence of neonatal sepsis according to the data from National Perinatal Database (NNPD) is 3 per 1000 live birth, 8.5 culture proven cases of sepsis per 1,000 live births and 40% of neonatal deaths were ascribed to sepsis in India[2].

Neonatal sepsis can be divided into two main classes depending on the onset of symptoms related to sepsis [3]; early onset sepsis usually presents within the first 72 hours of life and source of infection is generally the maternal genital tract. Late onset sepsis usually presents after 72 hours of age and source of infection is either nosocomial or community-acquired [3]. Risk factors associated with neonatal sepsis are Prematurity, low birth weight, prolong rupture of membrane >24 hours, premature rupture of membrane, febrile illness in the mother with evidence of bacterial infection within 2 weeks prior to delivery, foul smelling liquor, single unclean or >3 sterile vaginal examination(s) during labor, prolonged labor (sum of 1st and 2nd stage of labor >24 hours), perinatal asphyxia [4].

In general, gram negative bacteria are the predominant causes of neonatal sepsis and among them *Klebsiella pneumoniae* is the most common pathogen, especially in developing countries [2, 5, 6]

Development of antimicrobials resistance is more common due to inapparent use of antibiotics[7]. In India, drug resistance *K. pneumoniae* have emerged as important pathogens, causing fatal infections especially in context with neonates. Multi drug resistance (MDR), extensive drug resistance (XDR) and pan drug resistance (PDR) *K. pneumoniae emerged* due to various mechanisms; extended spectrum β-lactamase (ESBL), AmpC β-lactamase, Metalobetalactamase (MBL), Carbapenemase, 16S rRNA methylases, aminoglycoside modifying enzymes. There are limited data is available regarding drug resistance *K. pneumoniae* in neonates from central India.

We designed the present study to evaluate the occurrence of β-lacatamase enzymes as well as detect distribution of MDR, XDR and PDR in the *K. pneumoniae* from neonates at a tertiary care referral hospital in central India.

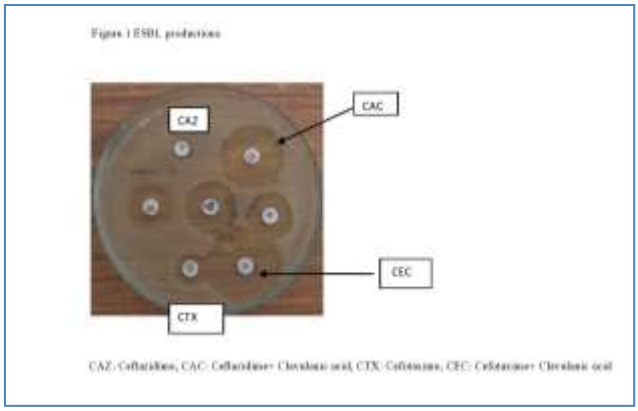
MATERIAL AND METHOD

The present study was conducted from February 2015 to July 2016 in Department of Microbiology Ruxmaniben Deepchand Gardi Medical College (R.D.G.M.C.) and Chandrikabahan Ruxmaniben Gardi, Hospital (CRGH), Ujjain (M.P.). During the study period, received blood samples in microbiology laboratory from clinically suspected neonatal sepsis patients and blood culture was performed. Briefly, 1–2 ml of blood was collected for culture into Automated BacT/ALERT blood culture bottles. Cultures were processed in BacT/ALERT 3D system (bioMe´rieux). These broths were incubated in system's incubator at 37°C under aerobic conditions for 5 days and observed for the growth of organisms. Signal of growth of bacteria given by machine was followed by sub-culture on MacConkey's agar and blood agar plates (HiMedia Laboratories, Mumbai) and identified using standard microbiological techniques [8-10].

Detailed history of neonates such as birth weight, sex and day of onset of sepsis were noted. Details regarding risk factors such as prolong rupture of membrane, pre term birth, birth asphyxia and difficult in resuscitation and history of antibiotic prior to onset of sepsis were also noted. Neonates with blood culture positive for *K. pneumoniae* were considered as cases and antibiotic susceptibility testing was done by Kirby–Bauer's disk diffusion method as per CLSI guidelines. Antimicrobial disks used were Amoxicillin-clavulanate (20/10µg), Piperaciln (100 µg), Piperacilin- tazobactum (100/10 µg), Cefepime (30 µg), Cefuroxime (30 µg), Cefoxitin (30 µg), Ceftazidime (30 µg), Cefotaxime(30 µg), Amikacin (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Cotrimoxazole (1.25/ 23.75 µg), Aztreonam (30 µg), Tetracyclin (30 µg), Imipenem (10 µg),Meropenem (10 µg) and Ertapenem (10 µg). Antimicrobial disks used in the study were procured from HiMedia Laboratories, Mumbai.

Detection of extended spectrum β-lactamase (ESBL) (Figure 1)

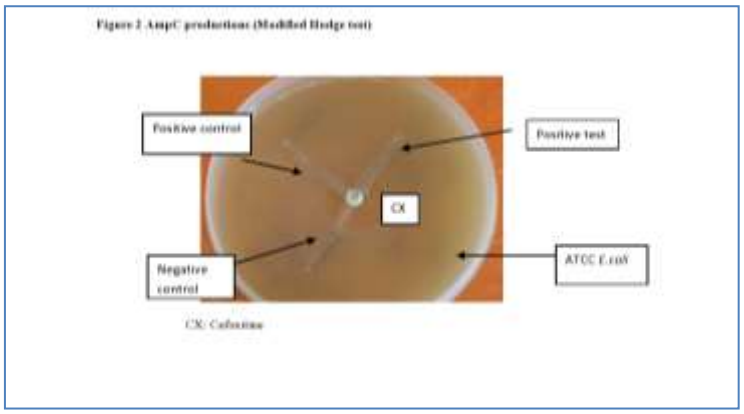
The screening for ESBL production was done as per recommended method [8]. Isolates shown zone of inhibition ≤ 22mm for Ceftazidime, ≤ 27 mm for Cefotaxime and ≤ 27 mm for Aztreonam was suspicious for ESBL production and isolates to be tested by a phenotypic confirmatory test combined disc diffusion method**.** Discs of Ceftazidime (30µg) alone and Ceftazidime-clavulanic acid (30µg/10 µg) are placed 20 mm apart from centre to centre on the agar plate. An increase of ≥ 5mm in zone inhibition with use of combination disc indicates the presence of ESBL. *Klebsiella pneumoniae* ATCC 700603 serve as quality control [8].



Detection of AmpC β-lactamase (Figure 2)

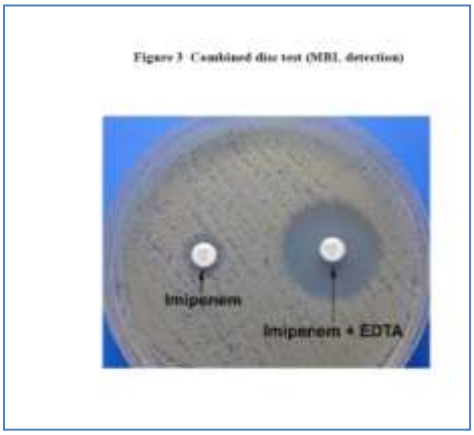
All the isolates were screened for AmpC β-lactamase by Kirby–Bauer's disk diffusion method using Cefoxitin (30 µg) disk. Zone of inhibition ≤ 18mm for cefoxitin was suspicious for AmpC production and is an indication for the organism to be tested by a phenotypic confirmatory test Modified Hodge Test[11, 12]. Broth suspension of a cefoxitin susceptible *E.coli* ATCC 25922 indicator strain was adjusted to 0.5 McFarland's standard and plated on Muller Hinton agar plate by using of sterile cotton swab. After drying, cefoxitin (30µg) disc was placed at

the centre of the plate and the test strains shown screening test positive streaked from the edge of the disc to the periphery of the plate. The plate was incubated overnight or 18-24 hours at 37^0C. Presence of a "diagonal" growth or 3mm or more in 'cloverleaf shaped' of zone of inhibition toward the test organism streak due to Amp C production by test strain was considered as positive. A negative Hodge test is shows with no diagonal growth into the cefoxitin zone [11, 12].



Detection of Metallo-β-lactamase (Figure 3)

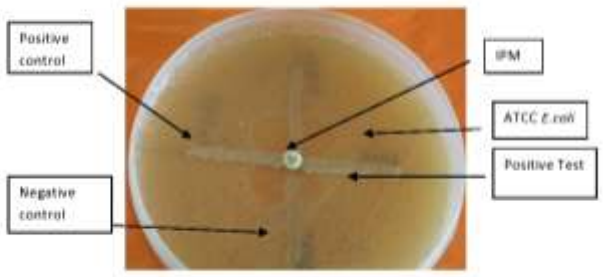
Isolates were resistant to carbapenemase (Imipenem, Ertapenam, Meropenam) and third generation cephalosporins (3GC) were considered screening positive. It is an indication for the organism to be tested by a phenotypic confirmatory test by combined disc test (Zone enhancement with EDTA-Imipenem disc) [8, 13]. Test organisms were inoculated onto plates of MHA. An Imipenem (10µg) disc and another Imipenem-EDTA disc were kept on the surface of the agar plate at the distance of 20 mm from centre to centre. The inhibition zones of Imipenem, and Imipenem-EDTA were compared after 16-18 hours of incubation in air at 35^0C-37℃. If increase in inhibition zone of imipenem and EDTA disc is ≥ 7mm than imipenem disc alone, test strain is considered to be the MBL producer [8, 10, 13].



Detection of Carbapenemase (figure 4)

Isolates were resistant to carbapenemase (Imipenem, Ertapenam, Meropenam) and third generation cephalosporins (3GC) were considered screening positive. It is an indication for the organism to be tested by a phenotypic confirmatory test by Modified Hodge test [8]. Culture suspension of *E. coli* ATCC 25922 adjusted to 0.5 McFarland standards and diluted 1:10in saline or broth was inoculated using a sterile cotton swab on the surface of MHA. After drying for 3- 10 minutes, 10µg Imipenem disc was placed at the centre of the plate. Using of sterile loop, picked 3-5 colonies of the test strain was inoculated in a straight line out from the edge of the disc to the periphery of the plate. The streak was at least 20-25 mm in length. The plate was incubated at 37^0C for 18-20 hours. Presence of a 'cloverleaf shaped' zone of inhibition due to carbapenemase production by test strain was considered as positive. *K. pneumoniae* ATCC BAA- 1705—MHT positive and *K. pneumoniae* ATCC BAA- 1706—MHT serve as negative controls [8].

Figure 4 Modified Hodge test (Carbapenemase detection)



IPM: Imipenem

RESULT AND OBSERVATION

During the study period 10.5% isolates were identified as *K. pneumoniae*. Out of that, 69% neonates were shown early onset neonatal septicemia and 31% late onset neonatal septicemia. 15% babies were born with mother had prolong rupture of membrane. 23% babies were preterm and low birth weight. 54% neonates had history of antibiotic use. Analysis of risk factor for *K. pneumoniae* is given in Table 1.

Isolates confirmed for β-lactamase enzymes including, ESBL positive 23.1%, for AmpC were 7.7%, Carbapenemase 46.1% and MBL 00%. In our study we recovered occurrence of co-production of AmpC and Carbapenemase were 7.7% (Table 2). Results of antimicrobial resistant pattern mentioned in figure 5.

In our study, total 23% isolates were found to be MDR, 77% isolates were XDR. None of the isolate was detected PDR as per guidelines (Table 3). 14

Table. 1. Analysis of risk factors for *K. pneumoniae* infection among neonates

Parameter *K.pneumoniae*	n=13	%age
PROM>24hrs	2	15
Perinatal asphyxia or difficult resuscitation	4	31
Birth weight ≤ 2.5 Kg	3	23
Full term vaginal delivery	10	77
Preterm babies	3	23
Lower segment caesarean section delivery	3	23
Male sex	7	54
Female sex	6	46
Early onset sepsis	9	69
Late onset sepsis	4	31
History of Antibiotic intake	7	54

Table 2. β-Lactamase production in *Klebsiella pneumoniae* isolates (n=13)

β-lactamase	Number of isolates (%)
ESBL	3 (23)
AmpC	1(7.7)
MBL	0(00)
Carbapenemase	6(46.1)
AmpC+ Carbapenemase	1(7.7)
Negative	4(31)

Table 3. MDR, XDR and PDR in *K. pneumoniae* isolates (n=13)

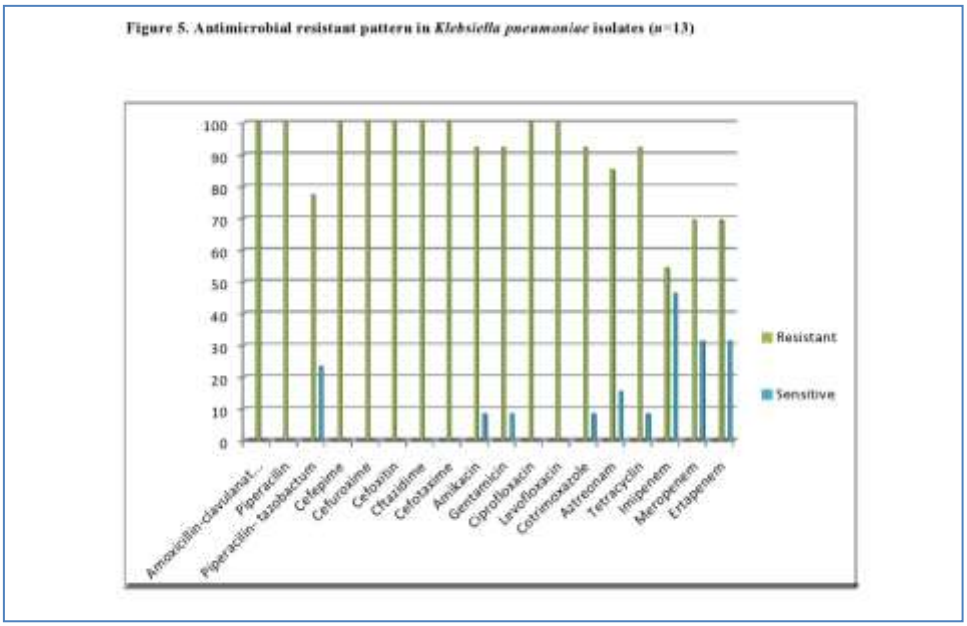
Antimicrobial category*	No. of Resistant	% of Resistant
MDR	3	23
XDR	10	77
PDR	00	00

*β-lactams (Penicillin/Cephalosporins), carbapenems, Aminoglycosides, fluoroquinolones +Monobactams+ Cotrimoxazole +Tetracycline.

MDR:[14] Non-susceptibile to≥1 agent in≥3 antimicrobial categories

XDR:[14] Non susceptibile to ≥1 agent in all but ≤2 antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories)

PDR: [14]Non-susceptible to all antimicrobial agents



DISCUSSION

Neonatal septicemia is the major problem worldwide and leading cause of neonatal morbidity and mortality. Our study presented an alarming MDR and XDR among *K. pneumoniae* in neonates. B-lactamase production among almost all isolates found. The widespread inappropriate use of antimicrobials especially higher antibiotics like carbapenem and cephalosporin are important risk factors of emergence of drug resistant superbugs [15].

During the study period 10.5% isolates were identified as *K. pneumoniae*. Out of that, 69% neonates were shown early onset neonatal septicemia and 31% late onset neonatal septicemia. As early sepsis is more common than late which is comparable with similar study held in Jaipur 78% and 22% and Pudducherri 87.9% and 12.1% respectively[16, 17].

We noted that, 15% babies were born with mother had prolong rupture of membrane (PROM). Similar finding also documented in Pudducherri 12.1% [17].

Pre term babies with sepsis in our study are lesser than others, 23% babies were preterm and low birth weight which is in contrast with finding from Jaipur 67.3% [16]. Pre term babies are more prone to sepsis but lesser reported in our study may be due to less sample size.

Antibiotic resistance pattern is varies centre to centre. In present study all isolates shown maximum resistance 100% to Amoxicillin-clavulanate, Piperacillin, Ceftazidime, Cefotaxime, Cefepime, Cefuroxime, Cefoxitin, Ciprofloxacin and Levofloxacin. 92% isolates were resistance for Amikacin. In general third generation cephalosporin (cefotaxime) and Amikacin use as first line drugs for empirical treatment in neonatal sepsis extensively, as a result they are rendered useless. In our study isolates were least resistance for Imipenem 54%. The rate of resistance to thease drugs was in co-ordinance with other studies [18, 19].

Regional variations have been detected in the distribution of ESBL producing isolates, and it is often a local problem. ESBL was detected in 23.1% of isolates, which is correlated with study by Brenden *et al.* 25%[20]. In contrast other study by modi 75.92%[18], which was higher than our study. On the other hand, the present study is higher than Shivali *et al*. 8.3% [19]. The high percentage of ESBL-producing isolates may be due to the selective pressure imposed by extensive use of third generation cephalosporins in the intensive-care unit. In our study, lesser number of ESBL positivity seen may be due to high-level expression of Carbapenemase or AmpC which can mask the phenotype detection of ESBL.

There is lack of data available regarding distribution of AmpC producing strains in India, and very little information available in neonatal groups. In our study AmpC producing isolates were 7.7%, comparable with result by Modi *et al*. 7.4% [18]. But it is slightly lower than study done by Shivali *et al*. 12.5% [19].

We recovered 46.1% of Carbapenemase producing *K. pneumoniae* isolates. This is consistence with study conducted in Assam 50% [21]. None of carbapenemase Isolates reported by other study [22]. Even though the existence of carbapenemase, its demonstration from neonates is extremely limited [23].

In present study, none of isolate was MBL positive may be due to excess production of carbapenemase and other mechanisms of carbapenem drug resistance. Geographically distribution of MBL varies, depends on local factors [19].

In our study, total 23% isolates were found to be MDR, In contrast with Shivali *et al*. reported 96%[19] Distribution of XDR among *K. pneumoniae* varies region to region due to local reasons. In present study 77% isolates were XDR, which is higher than report from China 10.7% [24].

Increased incidence of drug resistant strains observed in our study may be because our hospital is a tertiary care center in a rural setup and patients from adjoining districts and even villages are admitted for treatment. Which is may be a major reason of higher yielding of XDR isolates in present study. None isolate was detected PDR, which is concordance with various other studies [18, 19].

LIMITATION OF STUDY

Limitation of our study is that, Sample size was less and it is a single centre study. For proper evaluation of distribution of drug resistance mediated by different mechanisms, multi centre study needed. Molecular based specification for isolates was not done due to lack of facility.

CONCLUSION

The emergence and spreading of MDR and XDR *K.pneumoniae* due to β-lactamase production in neonates is high as the prevalence reported in other studies worldwide, however, antibiotic resistance remains one of the main challenging issues demanding for further attention. Good infection control practices, rational antibiotic policies, judicious use of interventions and implementation of standard of isolation precautions are of vital importance today. Unless there are strategies to optimize effective use of antibiotics, very few options will be left in future in the antibiotic armamentarium and it might herald an era of medical disaster with strains virtually untreatable with current spectrum of antimicrobials.

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