ejpmr, 2017,4(12), 433-435

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

NEONATAL SEPSIS DUE TO KLEBSIELLA: FREQUENCY, ANTIBIOTIC SUSCEPTIBILITY AND MOLECULAR CHARACTERIZATION BY 16S RRNA ANALYSIS

Sohail S. Khan*[1] and Budhlani G. N.[2]

[1]P. G. Department of Microbiology, Adarsh Mahavidhalaya, Dhamangaon Rly (M.S.).
[2]Adarsh Mahavidhalaya Dhamangaon.

***Corresponding Author: Sohail S. Khan**

P. G. Department of Microbiology, Adarsh Mahavidhalaya, Dhamangaon Rly (M.S.).

Article Received on 14/10/2017 **Article Revised on 15/11/2017** **Article Accepted on 26/11/2017**

ABSTRACT

Sepsis is a significant cause of morbidity and mortality in neonates. The focus of this study was to isolate and to identify *Klebsiella pneumoniae*, and its confirmation by 16s rRNA analysis. Present study revealed that *Klebsiella pneumoniae* is the commonest organism responsible for neonatal sepsis. One hundred and nineteen (119) neonates had positive blood cultures due to *Klebsiella* infection out of 1000 cases. Patients with *Klebsiella* septicemia were categorized into two groups of early and late-onset sepsis. Present study showed Ampicillin resistance in 97.5% isolates and Penicillin resistance in 96.6 % isolates. Above 50% isolates exhibited resistance against Erythromycin, Norfloxacin, Tetracycline and Ciprofloxacin. 97.5% isolates of *Klebsiella* spp. were found to be most sensitive to Imipenem. This study of *Klebsiella* spp. causing neonatal sepsis and their sensitivity pattern is useful so that guidelines can be prepared for empirical antibiotic therapy.

KEYWORDS: *Klebsiella*, early and late onset, antibiotic susceptibility, 16s rRNA analysis.

INTRODUCTION

Sepsis is normally defined as bacteraemia in combination with systemic inflammatory response syndrome, but there is no widely accepted definition for neonatal sepsis (Haque KN. 2005). Neonatal sepsis may be categorized as early-onset or late. Early onset sepsis (EOS), present within 48-72 hours of life. Onset is most rapid in premature neonates. Early-onset sepsis is associated with acquisition of microorganisms from the mother and source of infection is generally the maternal genital tract. Clinically, neonates are observed usually with respiratory distress and pneumonia. Presence of some prenatal risk factors has been associated with an increased risk of early onset sepsis (Kaftan H, Kinney JS, 1998). Late-onset sepsis occurs (LOS) after 72 hours of age. The source of infection is either nosocomial or community-acquired and neonates usually present with septicemia, pneumonia or meningitis (Baltimore RS., 1998: Wolach B. 1997).

According to recent data from National Neonatal Perinatal Database (NNPD) 2000, the incidence of neonatal sepsis has been reported to be 38 per 1000 intramural live births in tertiary care institutions (National Neonatology Forum, 2000). Septicemia is the commonest clinical category with an incidence of 24 per 1000 live births. Meningitis is diagnosed in 0.5 per 1000 live births. Neonatal sepsis is one of the common causes of neonatal mortality contributing to 23% of all neonatal deaths (National Neonatology Forum, 2000). *Klebsiella pneumoniae* is the most frequently isolated pathogen (31.2%), followed by *Staphylococcus aureus* (17.5%) among the intramural live births. Among extramural babies admitted for neonatal problems, *Klebsiella pneumoniae* is the commonest organism (36.4%), followed by *Staphylococcus aureus* (14.3%) and *Pseudomonas* (13.2%).

Neonatal nosocomial infections are an important cause of neonatal morbidity and mortality, however it's reporting in India has been non-uniform (Pawa *et al*., 1997). This may be due to the lack of surveillance work and scanty studies on this aspect. The reported incidence of nosocomial sepsis in India ranges from 1.5-37%. Although any pathogen may be acquired by the neonates in the hospital, *Klebsiella* is lately emerging as an important cause of neonatal nosocomial infection (Gupta *et al*., 1993).

Sepsis with Gram negative microorganisms are increasingly reported nowadays particularly in Asian countries (Joshi *et al*., 2000). The inadvertent use of broad-spectrum antibiotics has led to the emergence of multidrug resistant Gram-negative bacteria (Koksal *et al.*, 2001). *Klebsiella* species are of significant importance in this regard (Roilides *et al*., 2000).

MATERIALS AND METHOD

Our study is based on a prospective analysis of 1000 neonates who admitted to preterm unit and intensive care unit (ICU) in different hospitals of Akola city. All newborns admitted during the period of study with one or more symptoms/signs suggestive of sepsis with or without risk factors of sepsis were recruited into the study. Babies who had received antibiotics prior to presentation as well as those whose mothers had received antibiotics within one week prior to delivery were excluded from the study.

During a period of research work, neonates who admitted were investigated for early onset sepsis (0-7 days of age) and late onset sepsis (>7-90 days of age). For every neonate recruited, 2 ml of venous blood was collected in sterile tubes containing anticoagulant from a peripheral vein. The blood culture specimens were processed according to standard methods in the microbiology laboratory.

All blood cultures were incubated in brain heart infusion broth at 37°C and inspected daily for 3 days for presence of visible microbial growth by observing any of one of the following; turbidity, haemolysis, air bubbles (gas production) and coagulation of broth, otherwise the results were considered as negative for microbial growth. Subcultures were made during three successive days on nutrient agar, blood agar, and Mac Conkey's agar. The inoculated plates were incubated under aerobic conditions for 24 hrs. *Klebsiella* isolates obtained were identified by standard microbiological techniques, namely, colony characteristics, and biochemical properties including cultural characteristics, IMViC tests and carbohydrate utilization tests. For molecular level confirmation, multidrug resistant strains were sent for characterization by 16s rRNA analysis in Yaazh Xenomics DNA sequencing service, Madurai (Chennai Branch), Tamil Nadu, (India).

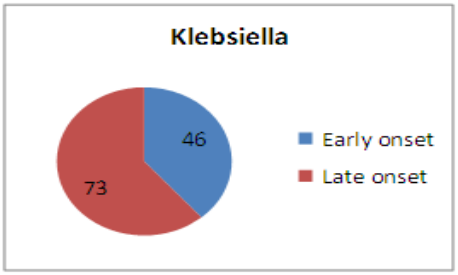
Simultaneously, by using Kirby-Bauer disc diffusion method (1966) antibiotic susceptibility pattern of the *Klebsiella* isolates was determined against Chloramphenicol, Ampicillin, Cefotaxime, Penicillin, Ceftazidime, Ceftizoxime, Ciprofloxacin, Erythromycin, Carbapenem, Norfloxacin, Imipenem, Gentamicin, Meropenem, Nalidixic acid, Tetracycline, Amoxyclav, Vancomycin, Amikacin, Furazolidone, Azithromycin.

RESULTS AND DISCUSSION

One hundred and nineteen (119) neonates had positive blood cultures due to *Klebsiella* infection out of 1000 cases. Patients with *Klebsiella* septicemia were categorized into two groups of early and late-onset sepsis Out of 119 cases, 46 (38.6%) were early onset sepsis (EOS) and 73 were late-onset sepsis (LOS).

Table 1: Frequency in early onset and late onset of *Klebsiella.*

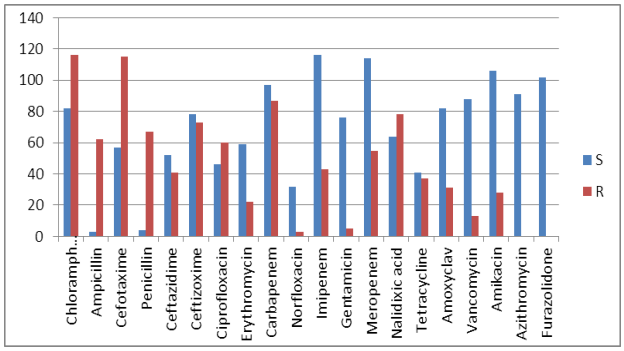
Organisms	Frequency N (%)	Early onset N (%)	Late onset N (%)
Klebsiella	119(16.1)	46(38.6)	73(61.3)



The antibiotic susceptibility pattern of *klebsiella* isolates was analysed against several antibiotics by disc diffusion method on Mueller Hinton Agar (Table 1). Present study showed Ampicillin resistance in 97.5% isolates and Penicillin resistance in 96.6 % isolates. Above 50% isolates exhibited resistance against Erythromycin, Norfloxacin, Tetracycline and Ciprofloxacin. 97.5% isolates of *Klebsiella* spp. were found to be most sensitive to Imipenem. These findings were similar to that reported by Missallati *et al.*, as they found *Klebsiella* isolates were resistant to Ampicillin. Similarly in the study of Roy *et al.*, Ampicillin resistance was observed in 100% isolates and 100% isolates of *Klebsiella* spp. were found to be sensitive to Imipenem and 40% isolates exhibited resistance against Erythromycin, Gentamicin, Tetracycline and Ciprofloxacin.

Table 2: Antibiotic activity against *Klebsiella* Spp.

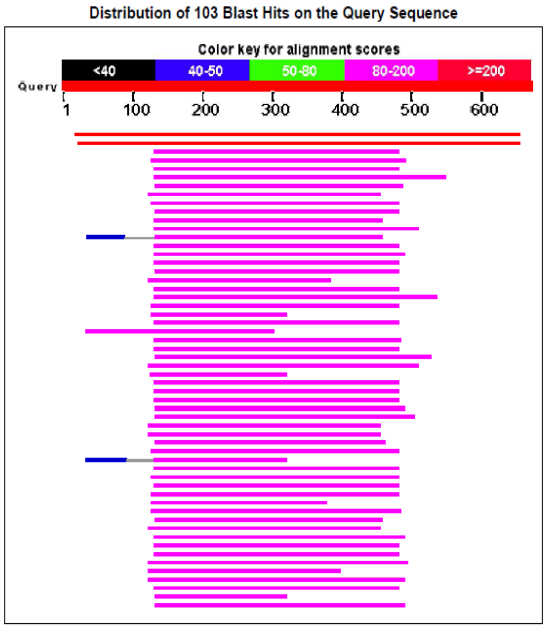
Antibiotics	*Klebsiella Spp.* n = 119			
	S%		R%	
Chloramphenicol	82	68.9%	37	31.1%
Ampicillin	3	2.5%	116	97.5%
Cefotaxime	57	47.9%	62	52.1%
Penicillin	4	3.4%	115	96.6%
Ceftazidime	52	43.6%	67	56.4%
Ceftizoxime	78	65.5%	41	34.5%
Ciprofloxacin	46	38.7%	73	61.3%
Erythromycin	59	49.5%	60	50.5%
Carbapenem	97	81.5%	22	18.5%
Norfloxacin	32	26.8%	87	73.2%
Imipenem	116	97.5%	3	2.5%
Gentamicin	76	63.8%	43	36.2%
Meropenem	114	95.8%	5	4.2%
Nalidixic acid	64	53.7%	55	46.3%
Tetracycline	41	34.5%	78	65.5%
Amoxyclav	82	68.9%	37	31.1%
Vancomycin	88	73.9%	31	26.1%
Amikacin	106	89%	13	11%
Azithromycin	91	76.5%	28	23.5%
Furazolidone	102	85.7%	17	14.3%



The primary identification of obtained isolates of *Klebsiella* spp. obtained was carried out based on cultural and biochemical characteristics. Alignment Search Tool (BLAST) data base (Altschul *et al.*, 1997) of National Center for Biotechnology (NCBI) Information was used to compare the sequence of 16S rDNA of the multidrug resistant strains with known 16S rDNA sequences of bacteria, with the help of Yaazh Xenomics, DNA sequencing service, Madurai (Chennai Branch), Tamil Nadu. The revealed sequence accession number is NC_016838.1 in NCBI; provided us taxonomy report with 94% similarity in BLAST analysis.

Table 3: Sequences producing significant alignments

Accession No.	Description	Total Score	Query Coverage	E value	Max Ident.
NC_016838.1	*Klebsiella pneumoniae* subsp. pneumoniae HS11286 plasmid pKPHS1, complete sequence	897	94%	0.0	93%



CONCLUSION

Study concluded that *Klebsiella* spp. was the predominant organism of neonatal septicemia, which was most frequently observed in late onset so it can be refer as hospital acquired infection and it was found to be almost 100% resistant to commonly used antibiotic Ampicillin and Penicillin whereas the highest sensitivity were observed against Imipenem, Meropenem and Amikacin. Molecular characterization by 16s rRNA analysis confirmed that neonatal septicemia majorly cause by the genus *Klebsiella pneumonia* pathogen.

REFERENCE

1. Baltimore RS.: "Neonatal nosocomial infections". *Semin Perinatol*, 1998; 22: 25-32.
2. Gupta P, Murali P, Murali MV, Faridi MMA, Kaul PB, Ramachandran VC, Talwar V.: "Clinical profile of *Klebsiella* septicaemia in neonates". *Indian J Paediatr*, 1993; 60: 565-572.
3. Haque KN.: "Definitions of bloodstream infection in the newborn". *Pediatr Crit Care Med*, 2005; 6(3Suppl): S45-49.
4. Joshi SG, Ghole VS, Niphadkar KB.: "Neonatal Gram-negative bacteremia". *Indian J Pediatr*, 2000; 67(1): 27-32.
5. Kaftan H, Kinney JS.: "Early onset neonatal bacterial infections". *Semin Perinatol*, 1998; 22: 15-24.
6. Koksal N, Hacimustafaoglu M, bagci S, Celebi S.: "Meropenem in neonatal severe infections due to multiresistant gram-negative bacteria". *Indian J Pediatr*, 2001; 68(1): 15-9.
7. Misallati A, El-Bargathy S, Shembesh N.: "Blood-culture-proven neonatal septicemia: a review of 36 cases". *East Mediterr Health*, 2000; (2-3): 483-86.
8. Pawa AK, Ramji S, Prakash K, Thirupuram S.: "Neonatal nosocomial infection. Profile and risk factors". *Indian paediatrics*, 1997; 34: 297-302.
9. Report of the National Neonatal Perinatal Database (National Neonatology Forum), 2000.
10. Roilides E, Kyriakides G, Kadiltsoglou I, Farmaki E, Venzon D, Katsaveli A, Kremeno poulos G.: "Septicemia due to multiresistant *Klebsiella* pneumonia in a neonatal unit: a case control study". *Am J Perinatol*, 2000; 17(1): 35-9.
11. Roy Subhasree, Rajni Gaind, Harish Chellani, Srujana Mohanty, Saswati Datta, Arun K. Singh & Sulagna Basu,: "Neonatal septicaemia caused by diverse clones of *Klebsiella pneumoniae* & *Escherichia coli* harbouring *bla*CTX-M-15", *Indian J Med Res* 137, April 2013; 791-799-791.
12. Wolach B.: "Neonatal sepsis: pathogenesis and supportive therapy". *Semin Perinatol*, 1997; 21: 28-38.