ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

AEROBIC BACTERIOLOGICAL PROFILE OF NEONATAL SEPTICAEMIA AND STUDY OF ESBL PRODUCTION IN GRAM NEGATIVE ISOLATES.

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Manuscript Info

Manuscript History:

Received: 18 September 2014
Final Accepted: 16 October 2014
Published Online: November 2014

Key words

extended spectrum of beta lactamases ,neonatal ,commonest ,septicaemia ,isolates .

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Abstract

Hundred clinically suspected septicaemic neonates ,admitted in neonatal intensive care unit (NICU) of Government General hospital ,Siddhartha medical college ,vijayawada were studied over a period of one year . Blood culture positivity was 35% , out of which in early onset sepsis, culture positivity was 19(54%) and in late onset sepsis culture positivity was 16(45.7%). out of total isolates gram positive pathogens constituted 20(62.5%), in which coagulase negative *staphylococci* and *staphylococcus aureus* were commonest organisms 9 (45%). out of 35 total bacteria isolates , gram negative organisms constitute 15(37.5%). Among the 15 gram negative isolates ,*Klebsiella Pneumoniae* was commonest (52%),followed by *Escherichiacoli* 3(20%),Pseudomonas aeruginosa 3(20%) and *Acinetobacter* 1(8%). *Staphylococcus aureus* was the commonest organism in gram positive and *Klebsiella Pneumoniae* is commonest among gram negative bacilli . In late neonatal septicaemia the commonest causative gram negative isolate was *Kebsiella Pneumoniae* .All gram positive cocci i.e. *Staphylococcus Aureus*,coagulase negative *Staphylococci* were 100% sensitive to vancomycin .All gram negative bacilli i.e. *Klebsiella Pneumoniae* ,*Escherichiacoli* ,*Psuedomonas* species and A*cinetobacter* were 100% sensitive to Imepenem,followed by Cefperazone 80% .Gram negative isolates 15 (75%) were resistant to third generation cephalosporins , and all of them were screened for ESBL production The confirmation of ESBL production was done by double disk synergy test (DDST). out of 8 K*lebsiella Pneumoniae* isolates screened for ESBL , 5(62.5%) were ESBL producers .out of 3 *Escherichia* coli , 2(75%) were ESBL producers and 3 pseudomonas species were ESBL producers.In the present study the total number of extended spectrum of beta lactamase (ESBL) production in gram negative bacilli in neonatal septicaemia is 10 (66.6%)

Introduction

Neonatal septicaemia is a major cause of morbidity and mortality in new born and its incidence , according to data from National perinatal database (NNPD 2002-03) is 30 per 100 live birth, contributing to19% of all neonatal deaths.The most common cause of death in neonatal period is infection (32%) followed by birth asphyxia(29%) and prematurity(30%). Neonatal septicaemia currently causes 1.6 million deaths in developing countries. Group B *streptococci* (GBS) and Coagulase negative *staphylococci* (CONS) are most common etiological agents for early onset and late onset neonatal septicaemia respectively(Kliegman , Behram,Nelson text book of paediatrics 18th edition).

In developing countries *Escherichia coli ,klebsiella spp, Acinetobacter* precede Group B *streptococcus* (GBS) and Coagulase negative *staphylococci* (CONS) in causing early onset neonatal septicaemia.*Klebsiella spp* and *Pseudomonas spp,Salmonella spp* and *Serratia* precede Coagulase Negative *Staphlococcus (*CONS) and *Staphylococcus aureus* in causation of LOS.(Chaudry Habibur et al 2007).The source of infection in early onset septicaemia (EOS) usually is the maternal genital tract .Infants with EOS,usually present with respiratory distress and pneumonia. The source of infection in late onset septicaemia(LOS) is either nosocomial or community acquired. The neonates usually present with pneumonia and meningitis in LOS.Blood cultures are considered as the gold standard for diagnosis of neonatal septicaemia.nevertheless,their positivity varies widely (50 to 87 %) and the results are not available rapidly. Neonatal septicaemia is a life threatening emergency .and its diagnosis is often difficult due to minimal signs and symptoms both in preterm and term infants .Delay in diagnosis and judicious use of antibiotics can bring down neonatal morbidity and mortality substantially .Surveillance is needed to identify the pathogens of neonatal septicaemia as well as antibiotic resistance pattern and there newer mechanisms of resistance . Among the beta lactamases ,Extended spectrum beta lactamases (ESBL) and Amp C beta – lactamases are most commonly produced.

MATERIAL AND METHODS

This prospective study was conducted on 100 neonates with signs and symptoms of septicemia admitted in neonatal intensive care unit, Government General Hospital/ Siddhartha Medical college, Vijayawada over a period of one year from January 2012 to December 2012 Informed consent was obtained from parents of neonates. Patient selection ,Inclusion criteria -

- Age less than 28 days.
- Greater than 30 weeks of gestation and full term babies With signs of septicaemia like lethargy, poorfeeding,irritability,fever,vomiting, abdominal distension,jaundice, respiratory distress,hypothermia cyanosis and convulsions.

Exclusion criteria -

- Extreme prematurity less than 30 weeks of gestation.
- Gross congenital anamolies.
- Undergone surgery.

2 ml of blood sample was inoculated in blood culture bottle containing 10 ml of Brain heart infusion broth for the culture isolation. Subculture was done after 18 hours .The bottles were kept and observed for turbidity,gas production, haemolysis or any colour change for seven consecutive days in case of absence of growth .Media used for sub culturing include Chocolate agar,Blood agar,MacConkey agar medium and PPA media.The isolates were identified by colony morphology ,Gram staining,motility , following biochemical reactions as per standard procedure.Anti microbial susceptibility testing was performed by Kirby Bauer disk diffusion method,commercially available Mueller Hinton agar culture medium and antibiotic disks (Himedia) were used as per (clinical laboratory standard institute 2012 (CLSI) recommendations)The drugs tested wereAmpicillin (10µg)Amoxicillin /Calvulanic acid (20/10µg) Piperacillin(100µg)Cefotaxime(30µg),Ceftiraxone(30µg) Ceftazidime(10µg) .Isolates were screened for ESBL production by using disk diffusion method for Cefotaxime ,Ceftazidime,Ceftriaxone, and Cefpodoxime placed on inoculated plates containing Muller Hinton agar according to CLSI recommendation.Isolates showing inhibition zones size of <22 mm with Ceftazidime(30 µg),< 25 mm with Ceftraixone(30µg)and <17 mm for Cefpodoxime were screened as potential ESBL producers *Escherichia coli* ATCC 25922 was used as a negative control and *Kiebsiella pneumoniae* ATCC 7006031013 was used as positive control for ESBL production .Confirmatory test for ESBL :Phenotypic confirmatory test for ESBL producers was carried out for all isolates that were screened positive for ESBL production by Double disc synergy method as described by(Jarier et al)Double disk approximation or double disk synergy method.In this test a disk of third generation cephalosporin was placed 30 mm from a disk of Amoxicillin-Clavulanic acid.Increase in inhibition zone diameter towards combination disks with clavulanic acid indicated Extended spectrum beta lactamases (ESBL).

RESULTS

Out of 100 blood samples from neonatal septicaemia 35(35%) were culture positive and 65(65%) were culture negative respectively .Out of 35 culture positive isolates early onset septicaemia were 19 and 16 were late onset septicaemia accounting for 54% and 46% respectively .Out of 35 culture positive isolates, 20(62.5%) were gram positive and 15(37.5%) were gram negative pathogens . Out of 20 gram positive isolates 9(45%) were coagulase negative *staphylococcus* followed by 9 (45%)*staphylococcus aureus* and 2(10%) *streptococcus spp* .Among gram negative pathogens,*klebsiella pneumonia* were 8(52%) followed by *Pseudomonas* 3(20%) and also *Escherichia coli* 3(20%) and 1(8%) is *Acinetobacter spp* . Among gram positive isolates , coagulase negative *staphylococcus* was sensitive to 78% Ampicillin,75% Amoxyclav,90%Ciprofloxacin,87% Ceftriaxone,76% Cefotaxim,80% Piperacillin, and 100% vancomycin .*Staphylococcus aureus* was sensitive to 59% ampicillin, 85% amoxicillin, 60% Ciprofloxacin, 75% Ceftriaxone, 76% Cefotaxim, 80% Piperacillin, and100%Vancomycin. .Antibiotic sensitivities for various gram negative isolates is as follows,*klebsiella spp* was sensitive to 67% Amoxyclav,,64%Ciprofloxacin,31% Ceftriaxone,33% Cefotaxim,75% Cefperazone,33% Piperacillin, 100% Imipenem,14% Gentamycin,.*Escherichia coli* was sensitive to78% Amoxyclav, 66% Ciprofloxacin, 44% Ceftriaxon,44%Cefotaxim, 65% Cefperazone,35% Gentamycin,56% Piperacillin,100% Imipenem. *Pseudomonas spp* was sensitive to 80%Amoxyclav 86% Ciprofloxacin,72% Ceftriaxon,75% Cefotaxim, 80% Cefperazone,20% Gentamycin,80% Piperacillin, 100% Imipenem.*Acinetobacter spp* was sensitive to, Ceftriaxone, Cefotaxim, Cefperazone, Piperacillin, Imipenem . Out of 8 isolates of *Klebsiella pneumoniae* ,5 were resistant to third generation cephalosporins,out of 3 *Eschericihia coli* 2 were resistant to third generation cephalosporins,out of 3 *Pseudomonas spp* 3 were resistant to third generation cephalosporins,1 A*cinetobacter spp* was resistant to third generation cephalosporins.All the isolates which are resistant to third generation cephalosporins were tested for production of ESBL by double disk diffusion method . Among 8 *klebisella pneumonia* isolates ,5 were ESBL producers ,followed by 2 out of 3 *Escherichia coli* isolates .All 3 *pseudomanas spp* were ESBL producers and 1 *acintobacter spp* was found not to be producing ESBL .

TABLE 1: CULTURE POSITIVITY IN NEONATAL SEPTICAEMIA

(n=100)

out of 100 blood samples from neonatal septicaemia 35(35%) are culture positive and 65(65%) are culture negative respectively.

CULTURE POSITIVE	**35(35%)**
CULTURE NEGATIVE	64(65%)
TOTAL NO	100(100%)

TABLE 2 : comparison of culture positivity and time of onset of septicaemia

TIME OF SEPSIS	**CULTURE POSITIVE ISOLATES**
early on set sepsis (EOS)	19(54%)
late onset sepsis (LOS)	16(45.7%)
total	35(100%)

Out of 35 culture positive isolates early onset septicaemia were 19 and 16 were late onset septicaemia accounting for 54% and 46% respectively.

TABLE -3:

	Ampicillin	**Amoxycl a-v**	Ciproflo- **xacin**	**Cefotaxim**	**piperacill i-n**	**Cefopera Zone**	Vancom - **ycin**	**Ceftriaxone**
CONS	78%	75%	90%	76%	80%	78%	100%	87%
Staphyloc **occusaureus**	59%	65%	85%	75%	76%	76%	100%	60%
Streptococcus species	54%	65%	87%	76%	85%	75%	100%	67%

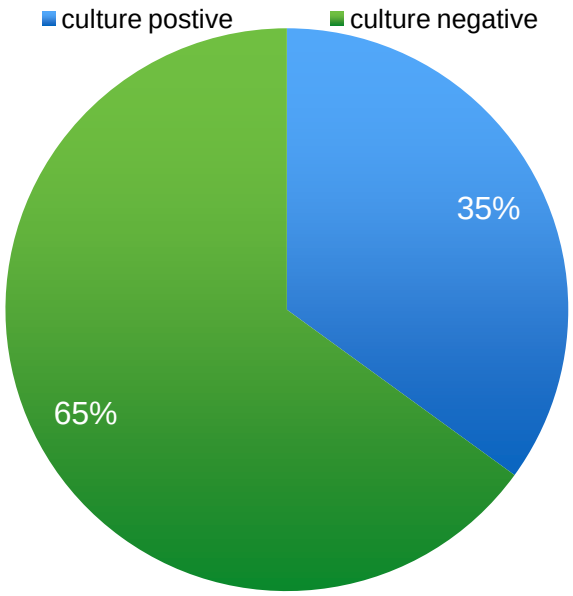
Antibiotics sensitive to gram positive organisms Antibiotic sensitivities for various gram positive isolates in our study is as follows, coagulase negative *staphylococcus* was sensitive to 78% Ampicillin,75% Amoxyclav,90%Ciprofloxacin,87% Ceftriaxone,76% Cefotaxim,80% Piperacillin, 100% Vancomycin.

Staphylococcus aureus was sensitive to 59% Ampicillin, 85% Amoxicillin, 60% Ciprofloxacin, 75% Ceftriaxone, 76% Cefotaxim, 80% Piperacillin, 100% Vancomycin.

Streptococci spp was sensitive to 54% Ampicillin, 66% Amoxyclav, 87% Ciprofloxacin, 67% Ceftriaxone, 76% Cefotaxim, 85% Piperacillin, 100% Vancomycin.

fig-1 NUMBER OF CULTURE POSITIVE SAMPLES

out of 100 blood samples from neonatal septicaemia 35(35%) are culture positive and 65(65%) are culture negative



respectively.

FIG 2: ANTIBIOTIC SENSITIVITY TO GRAM NEGATIVE BACTERIA.

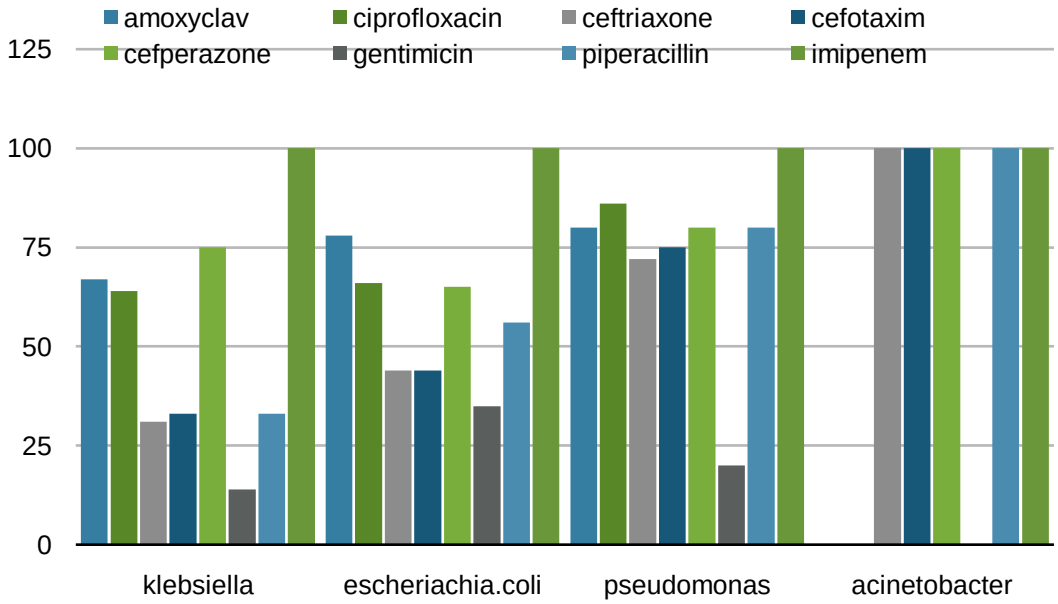


FIG 3: SCREENING OF ESBL IN GRAM NEGATIVE BACILL

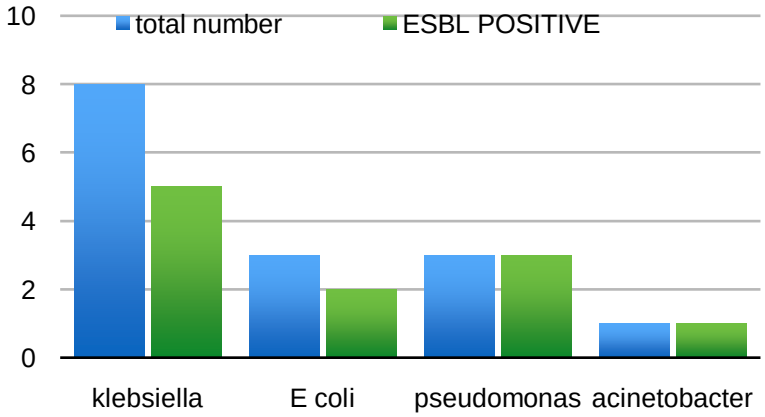


FIG 4: ESBL PRODUCTION IN SCREENED ISOLATES BY DOUBLE DISC DIFFUSION METHOD

FIG 5: ORGANISMS AMONG GRAM POSITIVE PATHOGENS

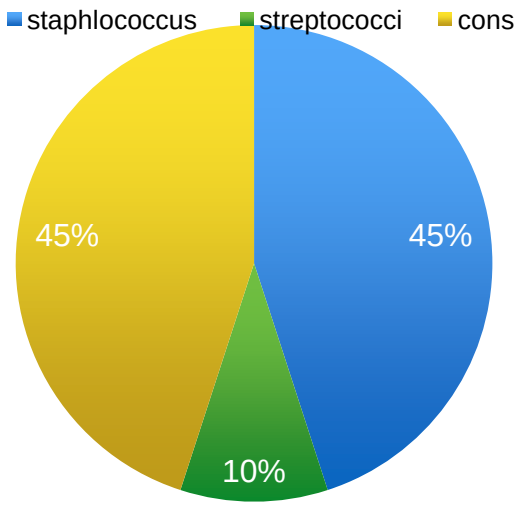
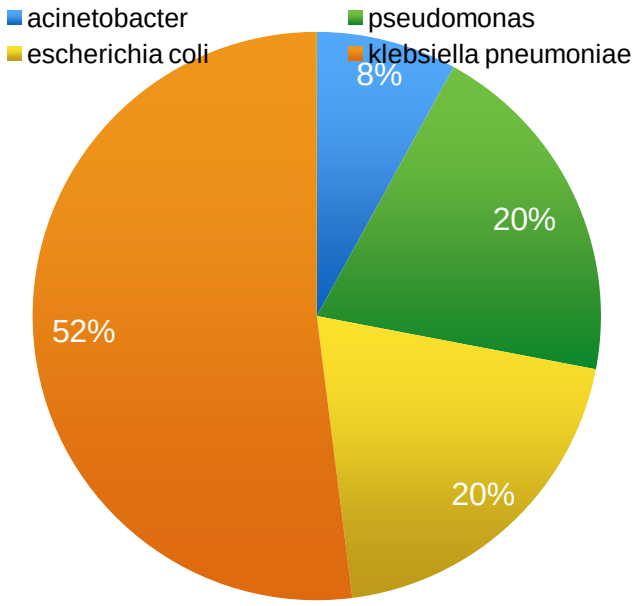


FIG-6 : GRAM NEGATIVE PATHOGENS IN NEONATAL SEPTICEMIA



DISCUSSION

Septicaemia is still a major cause of mortality and morbidity in neonates.Now a days Gram negative microorganisms are increasingly been reported as the major cause of neonatal septicaemia particularly in Asian countries.The emergence of multidrug resistant Gram-negative bacteria is mainly due to inadverent use of antibiotics.

The culture positivity in present study was 35 % and this coincides with Momtaz Mahesh ,et, al. (2004) (30%) and A.K.Mane, et ,al.(2010) 35% and Bhatacharjee, et,al. (2008) 48%.. Early onset septicaemia was observed in 54% and late onset septicaemia in 46 neonates .Early onset septicaemia was more common than late onset septicaemia which is compatible with the reports from Choudry Habibur, et, al. (2007) (70.7% EOS,30% LOS),A.H.Movahedion, et, al. (2006)(77.5%-EOS,22.5%- LOS) and vinod kumar, et,al. (2008) (55%-EOS,47%-LOS). .,Comparsion of Antibiotic resistant pattern in pathogenic isolates .Majority of Gram negative and Gram negative organisms isolated in present study were resistant to one or more antibiotics .This is in concurrence with other studies by Tallur ,et, al .(2000) and Kuruvilla, et ,al. (1998) .Present study revealed a very high degree of resistance of Gram negative bacilli not only to common used antibiotics ,but also predominantly to broad spectrum cephalosporins .These findings were compatible with other studies by Joshi ,et,al. (2000) and A.H Movahedian et, al.(2006).This is probably due to emergence of new variant existing strain as aresult of mutation or may be plasmid borne.In present study , Gram positive organisms were 100% sensitive to Vancomycin and Gram negative organisms were 100% sensitive to Imipenem.Similar findings were observed by Shaw Ck, et,al. (2007).In present study ,resistance rate of *Klebsiella pneumonia ,E.coli, Pseudomonas spp*, were 33 %, 44%, 30% repectively .*Acinetobacter spp* were 100 % sensitive to third generation Cephalosporins .These are similar to study by Ziba Mosayebi, et, al .(2003) reported that resistance rate of *Klebsiella pneumonia ,Escherichia coli ,Pseudomonas* were 40%,30%,20% respectively and *acinetobacter* were 100% sensitive to third generation Cephalosporins .In present study Gram negative isolates, resistant to third generation Cephalosporins were screened for ESBL production by Novel predictor Disk placement method ,ESBl production was further confirmed by Double Disc synergy Test(DDST) method as per CLSI guidelines .In present study ,ESBL production was observed in 62.5% of *Klebsiella* isolates ,75% of *Escherichia coli* isolates and 100% *pseudomonasspp*.These findings were similar to Bhattacharjee, et ,al .(2008)(ESBL production by *Klebsiella pneumonia* is 62.7%,E.coli 46.5%),B.V.Krishna ,et ,al .(2007) (ESBL production in *Klebsiella pneumonia* 71.93%) and Amita jain ,et, al .(2003) (ESBL production by

Klebsiella spp 86.6%,*E.coli* 63.6%), In the present study ESBL production in all Gram Negative Isolates was 66.6% comaprible to Savitha Jadhav, et ,al. (2012) Nashik, India (58%)and Leon Nicargua (72%).

CONCLUSION

The present study suggests that there is an increase in incidence of ESBL producers among Gram Negative pathogens i.e *Klebsiella pneumonia, Escherichia.coli,Pseudomonas spp and Acinetobacter* in neonatal septicaemia of both Early and late onset and many of them are resistant to commonly used third generation cephalosporins i.e cefotaxim,ceftriaxone and ceftazidime.Therefore Detection of Extended Spectrum Beta Lactamase (ESBL) production is of paramount importance both in hospital and community isolates. Clinicians should be familiar with the clinical significance of these enzymes and potential strategies for dealing with this as a growing problem. ESBL producing organisms pose a major problem for clinical therapeutics. Surveillance of patients of ICUs will help in early detection and control practices related to ESBL production.Antibiotic restriction and antibiotic cycling especially the empirical use of higher generation cephalosporins and carbepenems are other measures which if monitored properly, could help in control of the emergence and spread of ESBL producing bacteria.Hence paediatricians and Neonatologists, need to keep in mind that carbapenem must be kept in reserve for infections ,where other susceptible antibiotics can be used.

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