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Clinicomicrobial Profile of Neonatal Septicemia at a Tertiary Care Centre in Central India

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ABSTRACT

Neonatal septicaemia is an important cause of morbidity and mortality among neonates constituting about 30 – 50% of total neonatal deaths in developing countries. Data from various studies reveal that results obtained vary from place to place and from period to period. Early diagnosis and proper management of neonatal septicemia can reduce the morbidity and mortality substantially. 1) To study the bacterial etiologic agents responsible for neonatal sepsis. 2) To study the prevalent bacterial pathogens isolated from early onset neonatal sepsis (EOS) and late onset neonatal sepsis (LOS). 3) To study risk factors associated with neonatal sepsis. 4) To determine the susceptibility pattern of isolates to the commonly used antimicrobial agents in the treatment of sepsis. Blood culture reports were studied in 369 cases of clinically suspected septicemia in neonates using the standard conventional techniques. The antibiotic sensitivity was performed by Kirby-Bauer's disc diffusion method. In our study 66.93% had early onset sepsis and 33.06% had late onset sepsis. Male cases (63.95%) outnumbered female cases (36.04%). Premature rupture of membrane was the most common maternal risk factor (21.95%), while low birth weight was most common neonatal risk factor (70.73%). Blood culture reports were positive in 37.94 % of cases. *Klebsiella pneumonia* and *Enterococcus faecalis* were the commonest organisms causing neonatal sepsis in EOS while *Pseudomonas aeruginosa* and Coagulase negative staphylococcus species in LOS. Continued surveillance of neonatal sepsis should be done in order to follow closely changes in trends and risk factors, to obtain information for empiric antibiotic therapy and to react rapidly in case of major changes in susceptibility patterns and occurrence of outbreaks.

Keywords

Neonatal septicaemia, Antibiotic susceptibility testing.

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Introduction

Neonatal septicemia is an important cause of morbidity and mortality among neonates constituting about 30 – 50% of total neonatal deaths in developing countries.⁽¹⁾⁽²⁾Data from National Neonatal Perinatal Database(NNPD, 2002-03) reveal that the incidence of neonatal sepsis is 30 per 1000 live births,⁽³⁾sepsis develops in 20% neonates and 1% approximately die of sepsis related causes.⁽²⁾

Origin of Early onset sepsis (EOS) [within first week of life] is generally from pathogens of maternal genital tract, whereas late onset sepsis (LOS) [after first week till 28 days of life] is acquired either from the community or from hospital.⁽⁴⁾

Blood culture has a great diagnostic and prognostic significance in diagnosis of

neonatal septicemia. But a positive blood culture does not necessarily confirm infection, since blood contamination can occur.⁽⁵⁾ If intermittent bacteremia occurs, multiple site culture may help improve pathogen detection. However, single site blood culture with blood volume of >1ml can also detect neonatal sepsis.⁽⁶⁾

Data from various studies conducted over the last few decades reveal that results obtained vary from place to place and from period to period in which the studies were conducted.⁽⁷⁾ In the developing countries, *E. coli*, *Klebsiella species* and *S. aureus* being the most common pathogens of EOS, whereas *S. aureus*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* are the most commonly reported organisms in LOS. According to the National Neonatal perinatal Database of India, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *E. coli* are the three most common organisms causing neonatal sepsis both in hospital and community. Moreover, the causative organisms of EOS and LOS sepsis are similar especially in hospital setting in developing countries.⁽⁸⁾ The present study was therefore conducted to determine the microbial etiology and the antimicrobial susceptibility of the microbial isolates for effective management of the cases.

Materials and Methods

The study was carried out over a period of one year at a tertiary care setting after getting approval from the institutional ethical committee. Neonates admitted with diagnosis of neonatal septicemia were included in study. As per the criteria by Vergnono *et al.*,⁽⁷⁾ all the neonates presenting with convulsion, respiratory rate >60 breaths/min, severe chest indrawing, nasal flaring, grunting, bulging fontanel, redness around umbilicus extending to the skin, temperature 37.7°C or <35.5°C,

lethargic or unconscious, reduced movements, not able to feed, crepitations, cyanosis, reduced digital capillary refill time (>3 seconds) were included in the study.

Blood culture:⁽⁹⁾

Two blood samples from each neonate, 1-2 ml of blood were collected by standard collection procedure using proper aseptic precautions and inoculated immediately into 20 ml of brain heart infusion broth with 0.025% sodium polyanetholsulfonate as anticoagulant. Bottles were incubated at 37°C for 7 days. Three subcultures were made, first after 24 hr, and thereafter daily sub-culturing was done for 7 days on blood agar, MacConkey agar and chocolate agar. Identification of growth was by colony characters and standard biochemical tests.

Antibiotic sensitivity testing⁽⁹⁾ was performed by Modified Kirby-Bauer disc diffusion method⁽¹⁰⁾ as per CLSI recommendations.⁽¹¹⁾ Control strains used were *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212. Cefoxitin disc was used for testing methicillin resistance for Staphylococcal species.⁽¹¹⁾ For detecting high level aminoglycoside resistance for *Enterococcus* spp., gentamicin (120 µg) was used.⁽¹¹⁾

All the gram negative isolates were further tested for ESBL production using CLSI phenotypic confirmatory test.⁽¹¹⁾

CLSI phenotypic confirmatory test

ESBL was tested by applying the discs of ceftazidime and cefotaxime (30 µg) and ceftazidime/ cefotaxime and clavulanic acid (30 µg + 10 µg) to the lawn culture of the test organism. If the zone of inhibition around ceftazime/ ceftazidime clavulanic acid is ≥ 5 mm than the zone of inhibition around

ceftazidime disc, then the test organism is said to be ESBL producer.

Imipenem resistant *Pseudomonas* and *Acinetobacter* isolates were screened for metallo β lactamase production by combination disc test.⁽¹²⁾ A colony of the suspected isolate was suspended in Mueller Hinton broth and turbidity was adjusted to 0.5 McFarland opacity standards. Lawn culture was prepared on Mueller Hinton agar and combination disc test was put. The combinations used were imipenem (I) and imipenem –EDTA (I-EDTA). Imipenem (10 μ g) and combined imipenem/EDTA (750 mg) discs (Hi-media laboratories Pvt. Ltd., Mumbai) were placed on the agar plates. The test was considered MBL positive if $a > 7$ mm increase in the zone diameter for imipenem/EDTA was observed.⁽¹²⁾⁽¹³⁾

Results and Discussion

Total 369 neonates with neonatal septicemia were selected for present study. Early onset septicemia (0-72 hrs) was seen in 247 (66.93%) cases and late onset septicemia in 122 (33.06%) cases. Male preponderance was seen in both early onset AND late onset septicemia cases (Table 1). Among the maternal risk factors, premature rupture of membrane was seen in 81 (21.95%) cases. The most common neonatal risk factor observed was low birth weight 261 (70.73%), followed by prematurity 112 (30.35%) (Table 2). Of the 247 early onset neonatal septicemia cases, blood culture was positive in 84 (34%) cases. Similarly, out of 122 late onset neonatal septicemia cases, blood culture was positive in 56 (45.90%) cases (Table 3).

Gram negative bacilli were found to be commonest cause of neonatal septicemia (68.57%). Gram positive organisms were found in 39 (27.86%) cases. Five *Candida albicans* were isolated (Table 4). Among 87

blood culture positive early onset neonatal septicemia cases, gram negative bacilli (83.91%) were common aetiological agents as compared to gram positive cocci (16.09%). Among gram negative bacilli, 29 *Klebsiella pneumoniae*, 17 *Pseudomonas aeruginosa* and 14 *Acinetobacter baumannii* were the common isolates. Among gram positive organisms *Enterococcus faecalis* (9 isolates) was the commonest organism (Table 4). In 53 blood culture positive late onset neonatal septicemia cases, gram negative bacilli isolated were 23 (43.39 %) and gram positive cocci were 25 (47.17 %) while *Candida albicans* were isolated in 5 (9.44%) in number. Common bacterial isolates found in LOS were 18 coagulase negative *Staphylococci* followed by 9 *P. aeruginosa* (Table 4).

Among gram negative isolates, all the isolates were found to be sensitive to imipenem except for 2 isolates of *Acinetobacter* and *Pseudomonas* each. Maximum resistance was seen to amoxyclav, ceftazidime, cefotaxime. Among aminoglycosides, gentamicin was found to be most resistant (Table 5). Among gram positive isolates, maximum sensitivity was for vancomycin and linezolid. Penicillin was found to be the most resistant (except for *S. pneumoniae*) followed by erythromycin and clindamycin (Table 6). ESBL production was detected in 40 isolates (44.44%) by CLSI phenotypic method. Among these 40, 17 were *Klebsiella spp.*, 12 *Acinetobacter*, 6 *Pseudomonas* and 5 *E. coli*. Two isolates (imipenem resistant) each of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were investigated for metallo β lactamase (MBL) production. MBL was detected in one isolate each of *Pseudomonas aeruginosa* and *Acinetobacter spp.* MRSA was found in 3(37.5%) isolates and 5 (25%) methicillin resistant CoNS. Three isolates (37.5%) showed high level aminoglycoside resistance for *Enterococcus ssp* with gentamicin (120

µg) disc.

In this era of multidrug resistant organisms, neonatal septicemia remains an important challenging problem as it is associated with considerable morbidity and mortality. Identification of the microorganisms along with their antibiotic susceptibility pattern is one of the effective methods for proper management of neonatal septicemia cases.

Early onset septicemia (EOS) includes septicemia within less than 72 hrs of birth and is associated with presence of perinatal risk factors, maternal genital tract being the source of infection. However, late onset septicemia (LOS) includes septicemia of more than 72 hrs of birth and source of infection is either nosocomial or community acquired.⁽¹⁴⁾

In our study, of the 369 neonatal septicemia cases, early onset septicemia (0-72 hrs) was seen in 247 (66.93%) cases and late onset septicemia in 122 (33.06%) cases. Chugh *et al.*,⁽¹⁵⁾ had reported 68.8% cases of EOS and 32.2% cases of LOS. Movahedian *et al.*,⁽¹⁶⁾ also reported a higher percentage of early onset septicemia (77.5%) compared with late onset septicemia (22.5%). In our study, 236 (63.95%) male cases outnumbered 133 (36.04%) female cases. The male to female ratio is 1.77:1 (Table 1). Our finding is comparable with Khatua *et al.*,⁽¹⁷⁾ who observed male to female ratio 1.7:1. Khatua *et al.*,⁽¹⁷⁾ postulated that the factors regulating the synthesis of gamma globulins are probably situated on the X chromosome. Presence of one X chromosome in the male infant thus confers less immunological protection compared to female counterpart. Movahedian *et al.*,⁽¹⁶⁾ also reported a higher male: female ratio (2.64:1).

Among the maternal risk factors, premature rupture of membrane was seen in 81 (21.95%) cases (Table 2). Septicemia is more common

among those infants whose mother had PROM with increased risk of contamination of amniotic fluid by organisms from birth canal before delivery.⁽¹⁸⁾ The most common neonatal risk factor observed was low birth weight 261(70.73%), followed by prematurity 112 (30.35%) (Table 2). Khatua *et al.*,⁽¹⁷⁾ reported LBW in 79.3% cases. Movahedian *et al.*,⁽¹⁶⁾ reported 65% cases of LBW babies and 44.14% cases of prematurity.

Blood culture being the gold standard should be done in all suspected cases of sepsis prior to starting antibiotics. A positive blood culture and sensitivity of the isolate is the best guide to antimicrobial therapy.⁽¹⁹⁾ In the present study, blood culture positivity was seen in 37.94% and 62.06% were blood culture negative. Of the 247 early onset neonatal septicemia cases, blood culture was positive in 84 (34%) cases. Similarly, out of 122 late onset neonatal septicemia cases, blood culture was positive in 56 (45.90%) cases (Table 3). Murty *et al.*,⁽⁵⁾ reported a blood culture positivity of 52.63%.

Khatua *et al.*,⁽¹⁷⁾ reported 59.80% of blood cultures as positive. Mathur *et al.*,⁽²⁰⁾ reported blood culture positivity of 24.88%. In these studies and in the present study, there is a possibility of missing the anaerobes. In the present study, 62.06% blood cultures were negative and it does not rule out neonatal septicemia. These negative cultures may be due to antibiotic usage prior to sample collection, missed anaerobic infections.⁽⁵⁾

Gram negative bacilli were found to be commonest cause of neonatal septicemia (68.57%) and 27.86% were gram positive organisms (Table 4). Khatua *et al.*,⁽¹⁷⁾ reported 85% gram negative organisms and 15% gram positive organisms. Movahedian *et al.*,⁽¹⁶⁾ also reported a higher percentage of gram negative organisms (72.1%) as compared to 27.9% gram positive organisms.

The increased susceptibility of neonates to the gram negative bacteria may be due to the fact that the antibodies against these organisms are mainly IgM type. It is not passively transferred through placenta and is present at very low level in blood at birth, reaching the adult level by the age of 2 years. Adequate IgG (except IgG 2-subtype) levels at term,

afford protection against several gram positive bacteria.⁽²¹⁾

In blood culture positive early onset neonatal septicemia cases (87), gram negative bacilli (83.91%) were common aetiological agents as compared to gram positive cocci (16.09%).

Table.1 Age and sex distribution of neonatal septicemia cases (n=369)

Age	Male (%)	Female (%)	Total (%)
0-72 hrs (Early onset septicemia)	162 (65.58)	85 (34.41)	247 (66.93)
72 hrs – 4 wks (Late onset septicemia)	74 (60.65)	48 (39.34)	122 (33.06)
Total	236 (63.95)	133 (36.04)	369 (100)

Table.2 Maternal and neonatal risk factors for neonatal septicemia (n=369)

Risk factors	Number of septicemic neonates (%)
Maternal	
Premature rupture of membrane (PROM) > 24 hrs	81 (21.95)
Febrile illness in mother in last 15 days of delivery (Maternal fever)	66 (17.88)
Neonatal	
Low birth weight (weight < 2500 gm)	261 (70.73)
Prematurity (gestational age < 37 wk)	112 (30.35)
Neonatal resuscitation	99 (26.82)
Lack of breast feeding	56 (15.17)
Superficial skin infection including umbilical sepsis	47 (12.73)
Meconium aspiration	25 (6.77)

Table.3 Blood culture positivity in early onset and late onset septicemia (n=369)

Blood Culture	EOS (%)	LOS (%)	Total (%)
Positive	84 (34)	56(45.90)	140 (37.94)
Negative	163 (66)	66(54.10)	229 (62.06)
Total	247 (66.93)	122 (33.06)	369 (100)

Table.4 Microbial isolates from blood cultures of neonatal septicemia (n = 140)

Organisms	EOS (%)	LOS (%)	Total (%)
Gram positive organisms	14 (16.09)	25 (47.17)	39 (27.86)
<i>Staphylococcus aureus</i>	02	06	08
Coagulase negative <i>Staphylococci</i>	02	18	20
<i>Enterococcus fecalis</i>	08	01	09
<i>Streptococcus pneumonia</i>	02	00	02
Gram negative bacilli	73 (83.90)	23 (43.39)	96 (68.57)
<i>E. coli</i>	07	03	10
<i>Klebsiella spp.</i>	29	00	29
<i>Citrobacter freundii</i>	04	00	04
<i>Enterobacter aerogens</i>	00	04	04
<i>Salmonella typhi</i>	02	00	02
<i>Acinetobacter spp.</i>	14	07	21
<i>Pseudomonas aeruginosa</i>	17	09	26
Others		05 (9.44)	05 (3.57)
<i>Candida albicans</i>	00	05	05
Total	87 (62.14)	53 (37.86)	140 (100)

Table.5 Antibiotic resistance pattern of gram negative isolates (n=96)

Antibiotics	<i>E. coli</i> (n=10) (%)	<i>Klebsiella</i> <i>spp.</i> (n=29) (%)	<i>Citrobacter</i> <i>freundii</i> (n=4) (%)	<i>Enterobacter</i> <i>aerogens</i> (n=4) (%)	<i>S. typhi</i> (n=2) (%)	<i>Acinetobacter spp.</i> (n=21) (%)	<i>Pseudomonas</i> <i>aeruginosa</i> (n= 26) (%)
Amoxyclav	6(60)	29(100)	3(75)	3(75)	-	-	-
Piperacillin	6(60)	22(75.86)	3(75)	3(75)	-	17(80.95)	16(61.53)
Ceftazidime	7(70)	26(89.65)	3(75)	2(50)	-	16(76.19)	18(69.23)
Cefotaxime	6(60)	23(79.31)	3(75)	3(75)	0(0)	16(76.19)	21(80.76)
Cefepime	0(0)	10(34.48)	3(75)	1(25)	-	16(76.19)	19(73.07)
Piperacillin + tazobactam	1(10)	0(0)	1(25)	0(0)	-	5(23.8)	9(34.61)
Imipenem	0(0)	0(0)	0(0)	0(0)	-	2(9.52)	2(7.69)
Gentamicin	7(70)	23(79.31)	2(50)	3(75)	-	15(71.42)	16(61.53)
Amikacin	1(10)	15(51.72)	2(50)	2(50)	-	10(47.61)	10(38.46)
Tobramycin	4(40)	19(65.51)	1(25)	1(25)	-	15(71.42)	16(61.53)
Ciprofloxacin	8(80)	17(58.62)	2(50)	2(50)	0(0)	7(33.33)	9(34.61)

Table.6 Antibiotic resistance pattern of gram positive isolates (n=39)

Drugs	<i>Staph.aureus</i> (n=8)(%)	Coagulase negative <i>Staphylococci</i> (n=20) (%)	<i>Enterococcus</i> <i>faecalis</i> (n=8) (%)	<i>Streptococcus</i> <i>pneumoniae</i> (n=2) (%)
Penicillin G	8(100)	20(100)	8(100)	0(0)
Ampicillin	6(75)	15(75)	5(62.5)	1(50)
Cefoxitin	3(37.5)	5(25)	-	0(0)
Erythromycin	6(75)	5(25)	7(87.5)	0(0)
Clindamycin	6(75)	12(60)	-	-
Vancomycin (E-test)	0(0)	0(0)	0(0)	-
Linezolid	0(0)	0(0)	0(0)	0(0)
Gentamicin	4(50)	13(65)	3(37.5)	1(50)
Amikacin	5(62.5)	14(70)	3(37.5)	0(0)
Levofloxacin	5(62.5)	9(45)	4(50)	1(50)

Among gram negative bacilli, 29 *Klebsiella pneumoniae*, 17 *Pseudomonas aeruginosa* and 14 *Acinetobacter baumannii* were the common isolates. Among gram positive organisms *Enterococcus faecalis* (8 isolates) was the commonest organism (Table 4). In blood culture positive late onset neonatal septicemia cases (53), gram negative bacilli isolated were 23 (43.39 %) and gram positive cocci were 25 (47.17 %) while *Candida albicans* was isolated in 5 (9.44%) cases. Common bacterial isolates found in LOS were 18 coagulase negative *Staphylococci* followed by 9 *P. aeruginosa* (Table 4).

The bacteriological profile differs in EOS and LOS and it also differs in developing and developed countries. In developed countries gram positive organisms being predominant in both EOS and LOS, but Group B *streptococci* is more common in EOS. In developing countries gram negative organism are predominant in EOS and LOS both. ⁽²²⁾ Chugh *et al.*,⁽¹⁵⁾ reported 90.31% gram negative bacilli in EOS and 29% positive organisms in LOS. Stoll *et al.*,⁽²⁾ stated that majority of EOS 60.7% were caused by gram negative organisms. In a study by Guha *et al.*,⁽²³⁾ 1.6% *S. aureus*, 40% *E. coli*, 40%

Klebsiella pneumoniae, 16.2% *Pseudomonas spp.* from neonatal septicemia was reported. Mondal *et al.*, (19) reported 15.2% *Acinetobacter spp.* isolates. Mathur *et al.*,⁽²⁰⁾ also reported *Klebsiella pneumoniae* (38.5%) as the commonest organism isolated from neonatal septicemia, followed by 27.3% *Enterobacter spp.*, 11.15% *Pseudomonas spp.*, 6% *E. coli*, 10.25% *S. aureus*, 2.1% *S. epidermidis*, 0.9% *Acinetobacter spp.*. A study by Movahedian *et al.*,⁽¹⁶⁾ reported *Pseudomonas spp.*(36%) as the commonest organism isolated from neonatal septicemia, followed by 20.7% *S. epidermidis*, 17.1% *Klebsiella spp.*, 10% *Enterobacter spp.*, 6.3% *Citrobacter spp.*, 6.3% *S. aureus*, 2.7% *E. coli*.

With advancing medical and surgical management strategies, nosocomial fungal infections are also on an increase, *Candida spp.* being the most common nosocomial fungal pathogen and *C. albicans* is the most commonly isolated species (24). We isolated 9.44% *C. albicans* in our study (Table 4). Rani *et al.*,⁽²⁴⁾ isolated 3.74% *C. albicans*. Arora *et al.*,⁽²⁵⁾ isolated 4.73% cases *Candida spp.* in their studies. The life threatening illness, varying microbiological etiology of

neonatal septicemia and widespread antibiotic usage has resulted in antibiotic resistance. In our study, among gram negative isolates, all the isolates were found to be sensitive to imipenem except for 2 isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* each. Maximum resistance was seen to amoxycylav, ceftazidime, cefotaxime. Among aminoglycosides, gentamycin was found to be most resistant (Table 5).

Arora *et al.*,⁽²⁵⁾ had seen maximum resistance against ampicillin (89.96%), cephalexin (68.07%) and piperacillin (57.71%). Movahedian *et al.*,⁽¹⁶⁾ reports a very high degree of resistance among the gram negative organisms predominantly to broad spectrum cephalosporins. Gheibi *et al.*,⁽¹⁴⁾ also reported high resistance to cefotaxime (67.5%), ceftriaxone (65.3%) and ceftazidime (64.3%) among gram negative organisms but high sensitivity to amikacin (76.5%) and ciprofloxacin (92.8%).

Bhattacharjee *et al.*,⁽²⁶⁾ observed that more than 80% were sensitive to cefoperazone, amikacin, netilmicin, imipenem or piperacillin/tazobactam. Multidrug resistance was found in most of the gram negative bacilli strains in the present study.

Other workers have also reported multi drug resistance among the majority of gram negative isolates.⁽²⁵⁾ *Acinetobacter* spp. has emerged as an multidrug resistant superbug challenging its clinical management, particularly in medical and surgical intensive care units.⁽²⁷⁾ Bhattacharjee *et al.*,⁽²⁶⁾ reported that *P. aeruginosa* was sensitive to piperacillin/tazobactam (94%), imipenem (86%), cefoperazone (78%), ceftazidime (79%), ciprofloxacin (63%), amikacin (68%), gentamicin (53%) and tobramycin (38%).

Among gram positive isolates, maximum sensitivity was for vancomycin and linezolid.

Penicillin was found to be the most resistant (except for *S. pneumoniae*) followed by erythromycin and clindamycin (Table 6). Gheibi *et al.*,⁽¹⁴⁾ also reported maximum susceptibility of gram positive isolates to vancomycin, ciprofloxacin. Arora *et al.*,⁽²⁵⁾ reported the resistance of gram positive organisms to ampicillin (74.61 %) and erythromycin (69.67 %). Penicillin resistant stains, especially the hospital strains have increased to 75-90%. Most penicillin resistant staphylococcal strains act by producing β -lactamases hydrolysing the β -lactam ring.⁽²⁸⁾ Arora *et al.*,⁽²⁵⁾ reported Enterococcal drug resistance to ampicillin (93.33 %) and 86.67% resistance to erythromycin and linezolid.

Indiscriminate use of antibiotics has resulted in emergence of drug resistant strains. This has resulted in varying sensitivity pattern in different places and at times in same hospital at different times. Hence, it is very important to periodically review the strategies of antibiotic usage in neonates.

ESBL strains are associated with high mortality, being an important cause of nosocomial infection.⁽²⁹⁾ ESBL production was detected in 40 isolates (44.44%) by CLSI phenotypic method. Among these 40, 17 were *Klebsiella* spp., 12 *Acinetobacter* spp., 6 *Pseudomonas* spp. and 5 *E. coli*. Kim *et al.*,⁽²⁹⁾ reported ESBL production by 52.9% of *K. pneumoniae* and 17.9% of *E. coli* isolates.

Jain *et al.*,⁽³⁰⁾ have reported a high ESBL production by *Klebsiella* spp. (86.6%), *Enterobacterspp* (73.4%), *E. coli* (65.3%) strains and in none of the isolates of *Citrobacter* spp. The high percentage of ESBL producing *Klebsiella* spp. may be due to 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 for ESBL production.⁽³⁰⁾ In the present study, two isolates (imipenem resistant) each of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were investigated for metalloβ lactamase (MBL) production. MBL was detected in one isolate each of *Pseudomonas aeruginosa* and *Acinetobacter spp.* MRSA was found in 3(37.5%) isolates and 5(25%) methicillin resistant CoNS.

Three isolates (37.5%) showed high level aminoglycoside resistance for *Enterococcus ssp.* with gentamicin (120 µg) discs. Navneeth *et al.*,⁽³¹⁾ reported that 12 % *P. aeruginosa* were MBL producers. Lee *et al.*,⁽³²⁾ had reported 15.1% MBL production in imipenem resistant *Acinetobacter spp.* There are limited studies from the Indian subcontinent on MBL production by imipenem resistant *Acinetobacter spp.* Further studies on MBL production should be carried out by Clinical Microbiology laboratories.

Neonatal septicemia is a life threatening emergency and the single most important cause of neonatal deaths in the community accounting for over half of them. A coordinated effort to limit inappropriate use of broad-spectrum antibiotics, efficient hospital antibiotic policies, vigilant detection of resistant species rigorous surveillance and infection-control protocols are needed to control the increasing incidence of highly drug resistant organisms.

Strict infection control in neonatal units, hand washing combined with judicious policy for antibiotic therapy are the main solution to this problem. Continued surveillance of neonatal sepsis should be done in order to follow closely changes in trends and risk factors, to obtain information for empiric antibiotic therapy and to react rapidly in case of major changes in susceptibility patterns and occurrence of outbreaks.

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