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Bacteriological profile and antibiotic susceptibility of isolates from neonatal sepsis at neonatal intensive care unit (NICU) hospitals, Akola (M.S.)

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Abstract
Neonatal sepsis (NS) is a significant cause of mortality and morbidity in newborns. Neonatal sepsis refers to a clinical syndrome that is marked by signs and symptoms of infection in the first 28 days of life. Hence, the present study was undertaken to identify the common bacterial pahogens and to detect antibiotic susceptibility pattern of isolates for better treatment.
The present investigation is based on a prospective analysis of 738 suspected neonates admitted to neonatal intensive care unit (NICU) of different hospitals in Akola city, Maharashtra over a period from Jan 2014 to Jan 2017. In this study, 738 positive blood culture isolates were processed in accordance with standard laboratory techniques. Antimicrobial susceptibility of the isolates was done by Kirby Bauer disc diffusion method according to Clinical and laboratory standard institution (CLSI) recommendations.
Gram positive organisms (*Staphylococcus epidermidis, Staphylococcus aureus (CONS), Streptococcus agalecticus, Aerococcus* spp.) and Gram negative organisms (*Klebsiella Pneumoniae, E. coli, Proteus vulgaris, Pseudomonas* spp., *Acinetobacter* spp., *Enterobacter* spp. and *Salmonella* spp.) are the leading cause of neonatal sepsis in this study.
In present investigation, gram positive isolates exhibited high sensitivity against Imipenem and Meropenem followed by Amikacin, Gentamicin and Vancomycin where as high resistance was observed against commonly used antibiotics: Penicillin, Ampicillin, Norfloxacin and Amoxycillin. *S. epidermidis (*CONS) prevalent isolate amongst the gram positive organisms had high sensitivity to Imipenem, Meropenem and exhibited high resistance to commonly used antibiotics, Gram negative organisms had good sensitivity to Imipenem and Meropenem followed by Amikacin and Gentamicin. High resistance was noted against Penicillin, Ampicillin and Norfloxacin. *E. coli*, prevalent isolates amongst the gram negative organisms exhibited high susceptibility to Imipenem, Meropenem, Amikacin and Gentamicin.
It is concluded that all bacterial pathogens have emerged as the predominant pathogens responsible for neonatal sepsis which were resistant to commonly used antibiotics and multi drug resistant (MR). Results of this study suggest resistance pattern of microorganisms responsible for neonatal infections and are helpful to design a specific empirical antibiotic regimen.

Keywords: Neonatal septicemia, gram positive, gram negative, antibiotic susceptibility

1. Introduction
Neonatal sepsis (NS) is a significant cause of mortality and morbidity in the newborn [2]. Neonatal sepsis refers to a clinical syndrome that is marked by signs and symptoms of infection in the first 28 days of life, with or without isolation of a pathogen [25]. Incidence of neonatal septicemia in developed countries varies from 1-10/1000 live birth, whereas it is 3 times more common in India [24]. The incidence of neonatal sepsis according to the data from National Neonatal Perinatal Database (NNPD, 2002-03) is 30 per 1000 live births in India [14]. Neonatal sepsis is classified into the early-onset neonatal sepsis (≤72hrs) due to bacteria acquired before or during delivery, and late onset neonatal sepsis (>72 hrs-28days) involving pathogens acquired after delivery [20, 22].
Early onset sepsis is acquired during fetal life, delivery, or at the nursery [18]. Neonatal sepsis is caused by a variety of Gram positive as well as Gram negative bacteria, and sometimes yeasts [14]. Neonatal septicemia is one of the leading causes of neonatal mortality and morbidity worldwide.

Therefore, the present study was undertaken to isolate the bacteria commonly causing neonatal sepsis and determine antibiotic susceptibility pattern of blood cultures from neonates at a neonatal care units in Akola city (M.S.).

2. Materials and Methods

The present prospective analysis was carried out on 738 blood samples from neonates admitted to neonatal intensive care unit (NICU) at different hospitals of Akola city, Maharashtra from Jan 2014 to Jan 2017. All Newborns admitted during the period of study with one or more symptoms/sign suggestive of neonatal sepsis with predisposing risk factors were recruited into 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.

Using aseptic conditions, 2 ml. blood was drawn by a percutaneous venous puncture and inoculated into brain-heart infusion broth and incubated 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 negative for microbial growth. Subcultures were made on nutrient agar, blood agar, and MacConkey's agar. For confirmation again subcultures were made on selective and specific media. The identification of isolates was carried out based on cultural and biochemical characteristics by std. Microbiological techniques.

After the identification of bacteria, antimicrobial susceptibility testing of all blood culture isolates was done by Kirby- Bauer disc diffusion method on Muller Hinton agar as per Clinical and Laboratory Standards Institute (CLSI) guidelines [3]. Antibiotic susceptibility patterns of isolates were determined against Chloramphenicol, Ampicillin, Cefotaxime, Penicillin, Ceftazidime, Ceftizoxime, Ciprofloxacin, Erythromycin, Carbapenem, Norfloxacin, Imipenem, Gentamicin, Meropenem, Nalidixic acid, Tetracycline, Amoxyclav, Vancomycin, Amikacin, Furazolidone, Azithromycin.

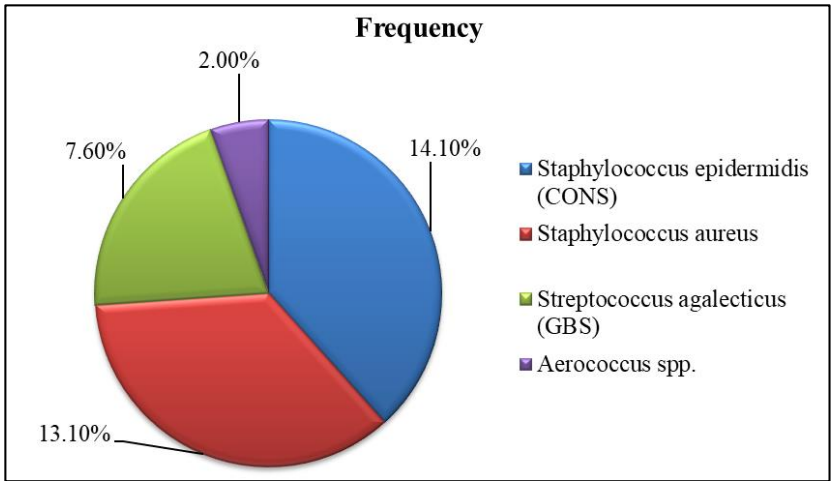
3. Results

The present study was conducted in nine different hospitals of Akola city of Maharashtra in which neonates admitted in NICU with signs and symptoms of sepsis were enrolled for study. Out of 1000 suspected blood samples collected from different hospitals, 736 (73.6%) were found to be culture positive. Among gram positive isolates, 207 (76.2%) isolates were found to cause early onset sepsis (EOS) whereas only 65 isolates were associated with late onset sepsis (LOS).

In present investigation, total four different types of gram positive organisms were isolated and identified. Details of these isolates are provided in Table 1 and Graph 1.

Table 1: Frequency Distribution of Gram Positive Bacterial Isolates.

Gm +ve Organisms	Frequency (n)	Percent
Staphylococcus epidermidis (CONS)	104	14.1%
Staphylococcus aureus	97	13.1%
Streptococcus agalecticus (GBS)	56	7.6%
Aerococcus spp.	15	2.0%
Total	272	36.9%

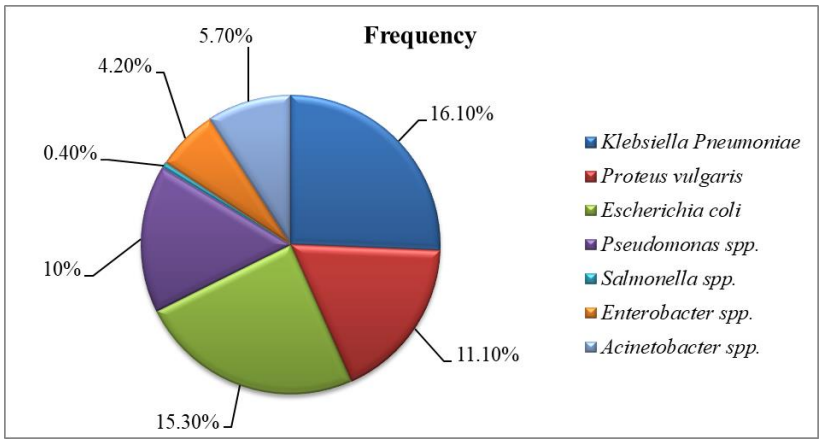


Graph 1: Frequency Distribution of Gram Positive Bacterial Isolates**.**

In present investigation, total seven types of gram negative organisms were isolated and identified. Details of these isolates are provided in Table 2 and Graph 2.

Table 2: Frequency Distribution of Gram Negative Bacterial Isolates.

Gm -ve Organisms	Frequency (n)	Percent
Klebsiella Pneumoniae	119	16.1%
Proteus vulgaris	82	11.1%
Escherichia coli	113	15.3%
Pseudomonas spp.	74	10%
Salmonella spp.	3	0.40%
Enterobacter spp.	31	4.2%
Acinetobacter spp.	42	5.7%
Total	464	63.1%



Graph 2: Frequency distribution of Gram negative bacterial isolates

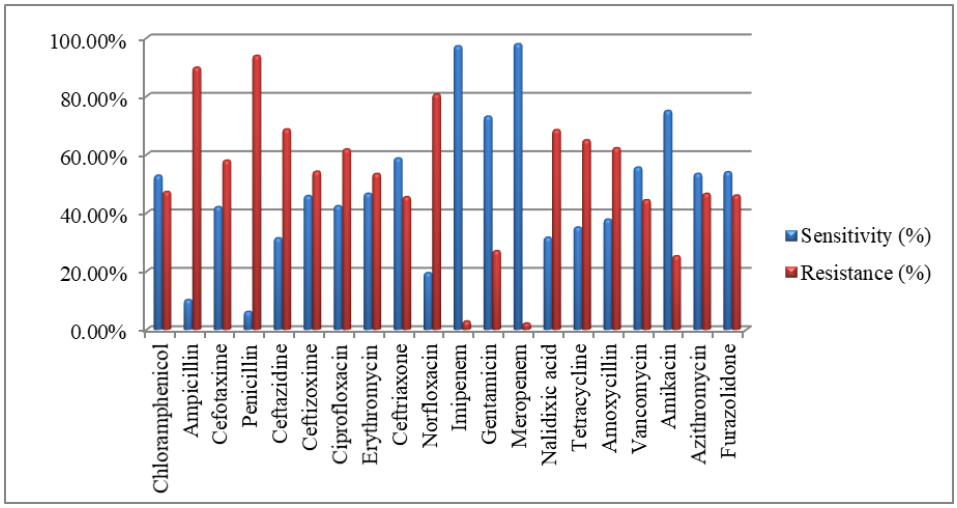
For effectual management of septicemia cases, study of bacteriological profile along with the antimicrobial sensitivity pattern plays a noteworthy role [5, 27]. Total 11 different types of organisms were isolated in which four types of gram positive isolates and seven types of gram negative isolates were obtained. The gram positive isolates were *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus agalecticus*, *Aerococcus* spp. and gram negative isolates were *Klebsiella Pneumoniae*, *E. coli*, *Proteus vulgaris*, *Pseudomonas* spp., *Acinetobacter* spp., *Enterobacter* spp. and *Salmonella* spp. *S*imultaneously, antibiotic susceptibility patterns of positive blood culture isolates against 20 different types of antibiotics, viz Chloramphenicol, Ampicillin, Cefotaxime, Penicillin, Ceftazidime, Ceftizoxime, Ciprofloxacin, Erythromycin, Carbapenem, Norfloxacin, Imipenem, Gentamicin, Meropenem, Nalidixic acid, Tetracycline, Amoxyclav, Vancomycin, Amikacin, Furazolidone were determined, using the Kirby Bauer disc diffusion method according to the Clinical and Laboratory Standard Institute [3].

Table 3, Graph 3 and Table 4, Graph 4 show the antibiotic susceptibility patterns in Gram positive and Gram negative isolates. Amongst the gram positive isolates, high sensitivity was observed against Imipenem and Meropenem followed by Amikacin, Gentamicin and Vancomycin. On the other hand, high resistance was observed against commonly used antibiotics such as Penicillin, Ampicillin, Norfloxacin and Amoxycillin.

Gram negative organisms showed good sensitivity to Imipenem and Meropenem followed by Amikacin and Gentamicin. High resistance was noted against Penicillin, Ampicillin and Norfloxacin.

Table 3: Antibiogram of Gram Positive Isolates

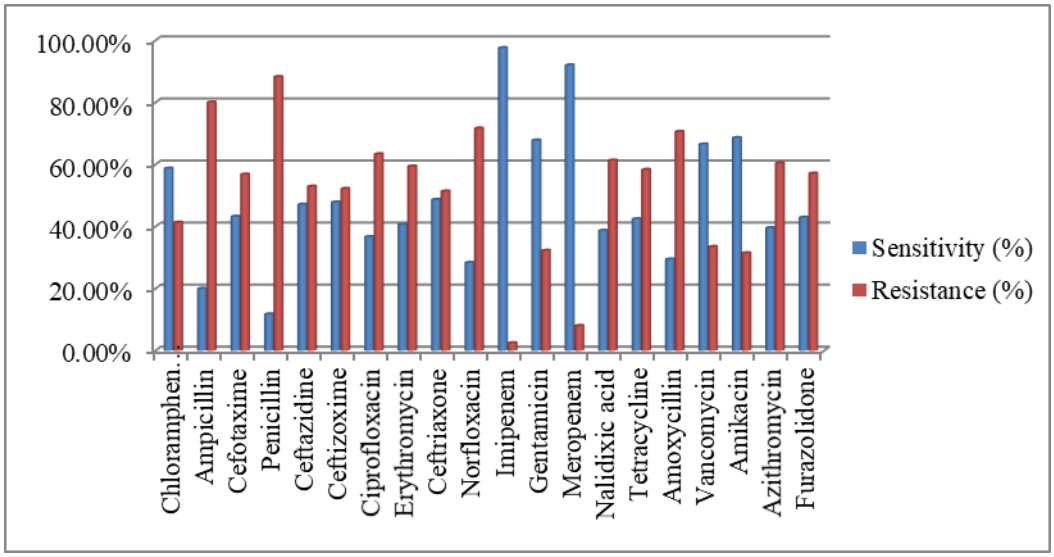
Antibiotics	Sensitivity (%)	Resistance (%)
Chloramphenicol	58.70%	41.30%
Ampicillin	19.98%	80.03%
Cefotaxime	43.20%	56.80%
Penicillin	11.75%	88.23%
Ceftazidime	47.10%	52.90%
Ceftizoxime	47.80%	52.20%
Ciprofloxacin	36.65%	63.35%
Erythromycin	40.60%	59.40%
Ceftriaxone	48.65%	51.35%
Norfloxacin	28.33%	71.65%
Imipenem	97.55%	2.45%
Gentamicin	67.78%	32.23%
Meropenem	92.00%	8.00%
Nalidixic acid	38.68%	61.30%
Tetracycline	42.40%	58.33%
Amoxycillin	29.43%	70.55%
Vancomycin	66.50%	33.50%
Amikacin	68.58%	31.43%
Azithromycin	39.55%	60.45%
Furazolidone	42.90%	57.10%



Graph 3: Antibiogram of Gram Positive Isolates

Table 4: Antibiogram of Gram Negative isolates

Antibiotics	Sensitivity (%)	Resistance (%)
Chloramphenicol	52.79%	47.21%
Ampicillin	10.17%	89.83%
Cefotaxime	42.04%	57.96%
Penicillin	6.11%	93.87%
Ceftazidime	31.33%	68.66%
Ceftizoxime	45.81%	54.17%
Ciprofloxacin	42.34%	61.79%
Erythromycin	46.60%	53.39%
Ceftriaxone	58.69%	45.44%
Norfloxacin	19.41%	80.59%
Imipenem	97.14%	2.84%
Gentamicin	73.03%	26.94%
Meropenem	97.87%	2.14%
Nalidixic acid	31.54%	68.44%
Tetracycline	35.03%	64.94%
Amoxycillin	37.74%	62.23%
Vancomycin	55.57%	44.41%
Amikacin	75.00%	25.17%
Azithromycin	53.41%	46.57%
Furazolidone	53.99%	46.00%



Graph 4: Antibiogram of Gram Negative isolates

4. Discussion

Gram negative and Gram positive septicemia was encountered in the culture positive cases in this study, which is comparable to a study conducted by researcher [1].

In present study, gram negative bacilli were more frequently (62.7%) involved in causing neonatal septicemia than gram positive cocci (37.3%) which was consistent with the studies done by [1, 11].

The results indicate that, gram negative organisms (51.3%, 238) has a preponderance in early onset sepsis (EOS) over the gram positive organisms (48.7%, 226) in late onset sepsis (LOS). Findings from this study corresponds to a study done in a Neonatal Intensive Care Unit (NICU) in Bangladesh, where they identified gram negative organisms (78%) to be the most common pathogen of neonatal sepsis [13]. The most predominant EOS causing organism was *Klebsiella pneumoniae* 73(61%) and *Escherichia coli* was the most common isolate identified of all the bacteria in early onset sepsis (EOS). Similar reports were given in a study done by researchers [13, 21]. Since reports indicate that, organisms causing EOS are mostly transmitted vertically from the colonized genital tract of mothers, or sometimes through the delivery process, the findings suggest that EOS causing organisms could be transmitted by these means [7].

Staphylococcus epidermidis (CONS) has been identified as the causative organism for EOS as proved in other studies [6, 23, 24]. In present investigation, among gram positive isolates the most predominant isolated organism was *Staphylococcus epidermidis* (CONS) 104 (14.1%) which is comparable to the findings of workers [4, 24]. The increasing prevalence of (CONS) infections is attributable to their increasing antibiotic resistance as reported by earlier researchers [16].

In view of the high morbidity and mortality associated with neonatal sepsis, the culture report cannot be awaited to administer antibiotics. Hence, area based knowledge of the bacteriological spectrum and their antibiotic sensitivity pattern is essential to formulate an empirical therapy [15].

E. coli, prevalent isolates amongst the gram negative organisms exhibited high rate of antibiotic susceptibility to Imipenem, Meropenem, Amikacin and Gentamicin as reported by investigators [20]. In the present study, *E.coli* isolated also exhibited a multi drug resistance to commonly used antibiotics, the majority of which were MR as reported

by researchers which poses a threat to neonatal care in this era of increasing antibiotic resistance [7].

S. epidermidis (CONS) prevalent isolates amongst the gram positive organisms showed high rate of antibiotic sensitivity to Imipenem, Meropenem as reported by investigators [20]. In the present study, high rate of antibiotic resistance of *S. epidermidis* (CONS) was observed to commonly used antibiotics, the majority of which were MR as reported by researchers [16].

5. Conclusion

Gram positive organisms(*Staphylococcus epidermidis, Staphylococcus aureus(CONS), Streptococcus agalecticus, Aerococcus* spp.*)* and Gram negative organisms(*Klebsiella Pneumoniae, E. coli, Protius valgaris, Pseudomonas* spp., *Acinetobacter* spp., *Enterobacter* spp. and *Salmonella* spp.) are the leading cause of neonatal sepsis in this study and It is concluded that most of them are resistant to commonly used antibiotics, multi drug resistant(MR) and these have emerged as the predominant pathogens responsible for neonatal sepsis. Results of this study suggest resistance pattern of microorganisms responsible for neonatal infections and are helpful to design a specific empirical antibiotic regimen.

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