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Neonatal sepsis: frequency and antibiotic susceptibility of pathogenic bacteria 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.

S. epidermidis (CONS) prevalent isolates amongst the gram positive organisms showed high rate of antibiotic sensitivity to Imipenem, Meropenem. In present investigation, antibiotic resistance of all isolates of *S. epidermidis (CONS)* was observed against Ampicillin and Penicillin. This study of *S. epidermidis (CONS)* causing neonatal sepsis and their sensitivity pattern is useful so that guidelines can be prepared for empirical antibiotic therapy.

Escherichia coli is a major cause of neonatal sepsis prevalent isolates amongst the gram negative organisms exhibited high rate of antibiotic sensitivity to Imipenem, Meropenem and Amikacin. In present investigation, *Escherichia coli* isolates also exhibited a multi-drug resistance to ampicillin, Penicillin, Nalidixic acid, Amoxicillin, Cefotexime, Norfloxacin and Tetracycline. This poses a threat to treatment of neonates due to increasing antibiotic resistance. It is concluded multi-drug resistant *E. coli* has emerged as the predominant pathogen responsible for early-onset neonatal sepsis, particularly in preterm infants. Hence, this study of *S. epidermidis* and *Escherichia coli* causing neonatal sepsis and their sensitivity pattern is useful so that guidelines can be prepared for empirical antibiotic therapy.

Keywords: Neonatal Septicemia, *Escherichia coli, Staphylococcus (CONS),* Antibiotic sensitivity.

1. Introduction

Neonatal sepsis (NS) is a significant cause of mortality and morbidity in the newborn [1]. 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 [20]. It is a major cause of neonatal mortality in the world, particularly in developing countries and is responsible for 30% - 50% of infant mortality in these communities [16]. At birth, the newborn is exposed to the microbial environment. NS can be categorized as early onset sepsis (EOS) and late onset sepsis (LOS). EOS is defined as onset of signs and symptoms of infection within 72 h of life and may be associated with pathogen isolation or not. In the LOS, signs and symptoms present after 72 h of life [19].

Escherichia coli is the most frequent Gram-negative organism that causes neonatal sepsis. *E. coli* is also a major neonatal sepsis pathogen worldwide, particularly in low-income countries [21]. Recent reviews of causative agents associated with infants with sepsis in the developing world revealed that in EOS, gram negative organisms predominated in the ratio of 2:1 with *Escherichia coli* being the most commonly isolated pathogen [22].

Coagulase-negative staphylococci (CONS) are the major pathogen involved in LONS (late-onset neonatal sepsis), particularly in infants born at a lower gestational age. According to more recent data from the National Institutes [10].

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CONS are common inhabitants of the skin and mucous membranes; although a small proportion of neonates acquire CONS by vertical transmission, acquisition primarily occurs horizontally [6, 20].

Neonatal septicemia is one of the leading causes of neonatal mortality and morbidity worldwide. Hence, the present study was undertaken to isolate the bacteria and determine their antibiotic susceptibility pattern of isolates for better treatment.

2. Materials and Methods

The present study is based on a prospective analysis of 738 neonates who admitted to preterm unit and intensive care unit (ICU) from Jan 2014 to Jan 2017 in the different hospitals of Akola city. All Newborns admitted during the period of study with one or more symptoms/ sign suggestive of sepsis with predisposing factors, 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 and inoculated into brain-heart infusion broth, 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 as negative for microbial growth. Subcultures were made on MacConkey's agar. The inoculated plates were incubated under specific conditions for 24 hr. For confirmation again subcultures were made on selective and specific media like Eosin Methylene Blue Agar. Isolates were identified by std. Microbiological techniques.

Simultaneously, antibiotic susceptibilities of *Escherichia coli* isolates to 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(CLSI) [2].

3. Results and Discussion

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.

The most predominant EOS causing organism was *Klesiella 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 [9,11]. In present investigation, among gram positive isolates the most predominant isolated organism was *Staphylococcus epidermidis* (CONS) about 104 (14.1%) which is comparable to the findings of workers [3, 12]. The increasing prevalence of (CONS) infections is attributable to their increasing antibiotic resistance as reported by earlier researchers [12]. In present investigation, high resistance of

all isolates of *S. epidermidis (CONS)* was observed against Ampicillin as observed in the previous study (Mustafa) [11] and Penicillin comparable to results reported in the study [12].

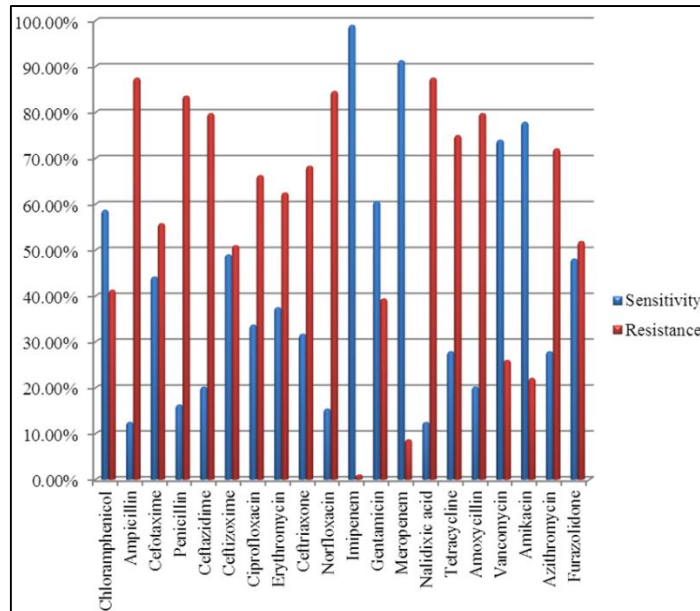**Table 1:** Antibiogram of *Staphylococcus epidermidis* (CONS)

Antibiotics	S. epidermidis (CONS) n=104			
	Sensitivity	Percentage	Resistance	Percentage
Chloramphenicol	61	58.7%	43	41.3%
Ampicillin	13	12.5%	91	87.5%
Cefotaxime	46	44.2%	58	55.8%
Penicillin	17	16.3%	87	83.6%
Ceftazidime	21	20.2%	83	79.8%
Ceftizoxime	51	49.0%	53	51.0%
Ciprofloxacin	35	33.7%	69	66.3%
Erythromycin	39	37.5%	65	62.5%
Ceftriaxone	33	31.7%	71	68.3%
Norfloxacin	16	15.4%	88	84.6%
Imipenem	103	99.0%	1	1.0%
Gentamicin	63	60.6%	41	39.4%
Meropenem	95	91.3%	9	8.7%
Nalidixic acid	13	12.5%	91	87.5%
Tetracycline	29	27.9%	78	75.0%
Amoxycillin	21	20.2%	83	79.8%
Vancomycin	77	74.0%	27	26.0%
Amikacin	81	77.9%	23	22.1%
Azithromycin	29	27.9%	75	72.1%
Furazolidone	50	48.1%	54	51.9%

In addition, it was observed that all the isolates of *S. epidermidis (CONS)* were uniformly sensitive to Amikacin and Vancomycin where as high rate of resistance was found against Nalidixic acid, Norfloxacin, Amoxicillin, Ceftazadime, Tetracycline and Azithromycin, Ciprofloxacin, Erythromycin, Cefotaxime, Furazolidone and Ceftixozime (Table 1,Graph 1 and Photoplate1). The increasing trend in the prevalence of MR-(CONS) has also been reported in other studies [1, 5, 11].

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 [9, 16]. *E. coli,* second among the isolated organisms in the present study, isolates were subjected to antimicrobial susceptibility and resistance pattern study against 20 different antibiotics. Details of results are provided (Table 2, Graph 2 and photoplate 2).

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 [11].

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 [15]. 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 [4]. High resistance was observed to Ampicillin, Penicillin and Nalidixic acid, Amoxicillin, Cefotexime, Norfloxacin and Tetracycline (Table 2, Graph 2 and photoplate 2). These results are consistent with a similar study done in Indonesia [21].

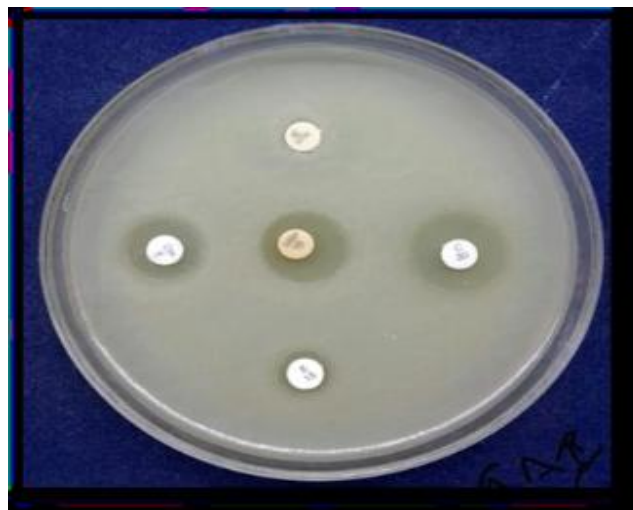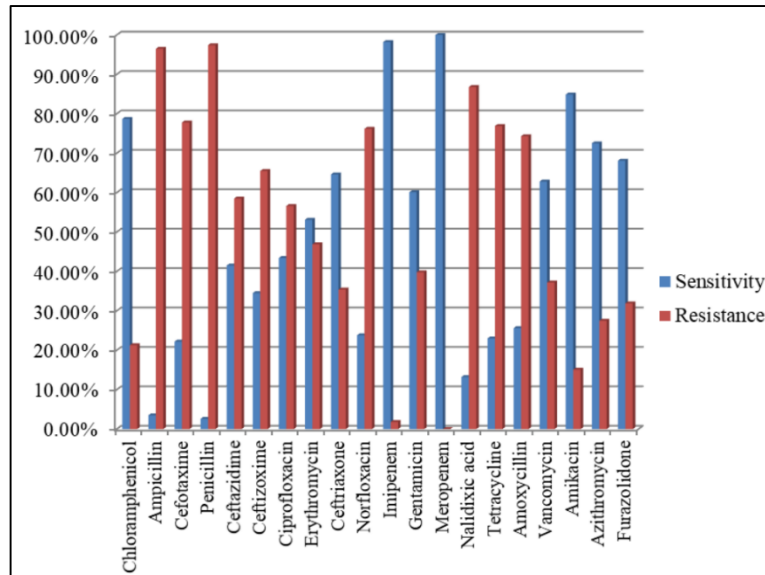**Graph 1:** Antibiogram of *Staphylococcus epidermidis* (CONS)

Plate 1: *Staphylococcus epidermis* showing susceptibility to C, CIP, E and MRP.

Table 2: Antibiogram of *E. coli*

| Antibiotics	*E. coli* n=113			
	Sensitivity	Percentage	Resistance	Percentage
Chloramphenicol	89	78.7%	24	21.3%
Ampicillin	4	3.5%	109	96.5%
Cefotaxime	25	22.2%	88	77.8%
Penicillin	3	2.6%	110	97.4%
Ceftazidime	47	41.5%	66	58.5%
Ceftizoxime	39	34.5%	74	65.5%
Ciprofloxacin	49	43.4%	64	56.6%
Erythromycin	60	53.1%	53	46.9%
Ceftriaxone	73	64.6%	40	35.4%
Norfloxacin	27	23.8%	86	76.2%
Imipenem	111	98.2%	2	1.8%
Gentamicin	68	60.1%	45	39.8%
Meropenem	113	100%	0	0%
Nalidixic acid	15	13.2%	98	86.8%
Tetracycline	26	23%	87	76.9%
Amoxycillin	29	25.6%	84	74.3%
Vancomycin	71	62.8%	42	37.2%
Amikacin	96	84.9%	17	15.1%
Azithromycin	82	72.5%	31	27.5%
Furazolidone	77	68.1%	36	31.9%

Graph 2: Antibiogram of *E. coli.*
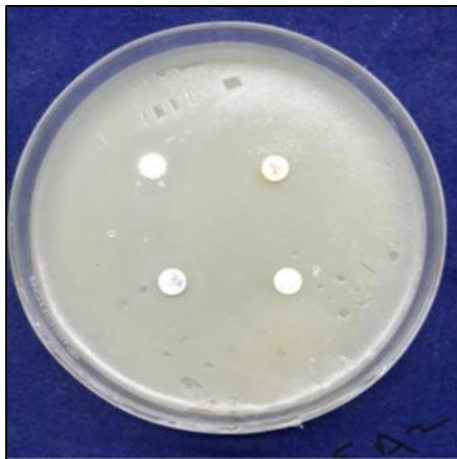
Plate 2: *E. coli* showing resistance to IE, CAZ, CTX and PEN.

4. Conclusion

Staphylococcus epidermidis (CONS) predominant organism of neonatal septicemia was the leading causative agent in late onset sepsis (LOS). It was reported as hospital acquired infection and found to be almost resistant to commonly used antibiotics, Ampicillin and Penicillin. High rate of sensitivity was observed against Imipenem, Meropenem and Amikacin. This Resistance pattern of microorganisms responsible for neonatal infections is helpful to design a specific empirical antibiotic regimen.

Escherichia coli major cause of neonatal sepsis prevalent amongst the gram negative organisms exhibited high rate of antibiotic sensitivity to Imipenem, Meropenem and Amikacin. *Escherichia coli* isolates exhibited a multi-drug resistance to Ampicillin, Penicillin, Nalidixic acid, Amoxicillin, Cefotexime, Norfloxacin and Tetracycline which poses a threat to treatment of neonates due to increasing antibiotic resistance. This resistance pattern of *Escherichia coli* responsible for neonatal infections is helpful to design a specific empirical antibiotic regimen.

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