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Neonatal sepsis due to *Escherichia coli*: Frequency, antibiotic susceptibility and molecular characterization by 16s RRNA analysis

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Abstract
Neonatal sepsis (NS) is a significant cause of mortality and morbidity in newborns. The present study is based on a prospective analysis of 113 out of 1000 neonates who admitted to preterm unit and intensive care unit (ICU) in different hospitals of Akola city from Jan 2014 to Jan 2017. The focus of this study was to isolate, to identify *Escherichia coli* isolates, its confirmation by 16s rRNA analysis and to detect antibiotic sensitivity pattern. *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. Hence, this study of *Escherichia coli* causing neonatal sepsis and their sensitivity pattern is useful so that guidelines can be prepared for empirical antibiotic therapy. Molecular characterization of multi drug resistant *Escherichia coli* isolates by 16s rRNA analysis was confirmed. It is concluded multi-drug resistant *E. coli* has emerged as the predominant pathogen responsible for early-onset neonatal sepsis, particularly in preterm infants.

Keywords: Neonatal septicemia, *Escherichia coli,* 16s rRNA

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 [18]. 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 [12]. 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 [16].
Escherichia coli is the most frequent Gram-negative organism that causes neonatal sepsis [5]. *E. coli* is also a major neonatal sepsis pathogen worldwide, particularly in low-income countries [17]. 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 [20]. However, the main causative agent of LOS in neonatal intensive care unit has been reported to be coagulase- negative staphylococcus (CoNS).
Due to the consumption of antibiotics over time and in different countries (developed or developing), changes in prevalence and sensitivity of these bacteria have occurred; for example, resistant bacterial strains include gentamicin-resistant *Klebsiella* species, third-generation cephalosporin-resistant gram-negative organisms, and methicillin-resistant staphylococci [6, 8]. Therefore, continuous epidemiologic monitoring by repeated local revisions of susceptibility patterns to antibiotic agents is necessary to establish a rational treatment strategy [3].

Hence, the present study was undertaken to isolate the bacteria, *Escherichia coli* causing neonatal sepsis and determine their antibiotic susceptibility pattern of *Escherichia coli* isolates from blood cultures of neonates at a neonatal care unit in Akola city.

2. Materials and Methods

The present study is based on a prospective analysis of 113 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 [4]. Performance Pure colonies of isolates were inoculated on Muller Hinton agar. Antimicrobial discs were placed on the inoculated agar and incubated for 24 h at 37 °C; they were then observed for zones of inhibition, breakpoints identified and determined as susceptible, intermediate or resistant according to the Clinical and Laboratory Standard Institute [4]. 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).

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 blood samples collected from different hospitals, 736 (73.6%) were found to be culture positive.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 [9]. 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, 14]. 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].

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 1 and Graph 1).

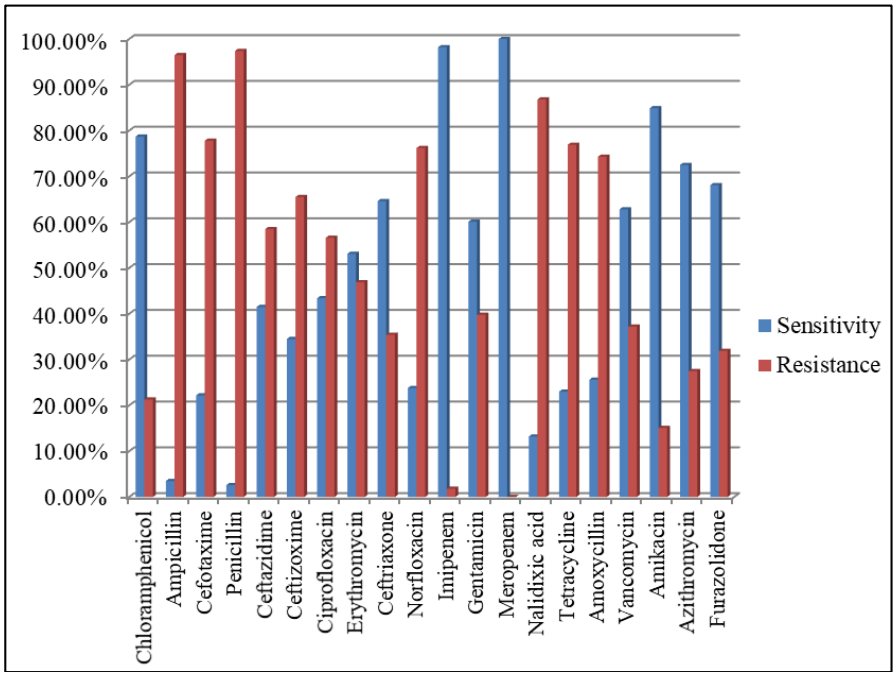
In view of the high morbidity and mortality associated with neonatal sepsis, the culture report cannot be awaited to administer antibiotics. Hence, area based knowledge of the bacteriological spectrum and their antibiotic sensitivity pattern is essential to formulate an empirical therapy [11].

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 [13]. 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]. High resistance was observed to Ampicillin, Penicillin and Nalidixic acid, Amoxicillin, Cefotexime, Norfloxacin and Tetracycline (Table 1,Graph 1). These results are consistent with a similar study done in Indonesia [19].

The primary identification of obtained ***Escherichia coli*** isolates of obtained was carried out based on cultural and biochemical characteristics (Photoplate 1).The Basic Local Alignment Search Tool (BLAST) data base of National Center for Biotechnology (NCBI) information was used to compare the sequence of 16S rRNA of the multidrug resistant strains with known 16S rRNA sequences of bacteria, with the help of Yaazh Xenomics, DNA sequencing service, Madurai (Chennai Branch), Tamil Nadu. Furthermore, the phylogenetic tree of the partial 16S ribosomal RNA genes sequence of *Escherichia coli* Isolate matched with 99% with the reference sequence of *Escherichia coli* in BLAST data base with the Accession No. LC056477.Thus, *Escherichia coli* Isolate was identified as a strain of *Escherichia coli* (Fig.1, Fig.2 and Table 2).

Table 1: 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 1: Antibiogram of *E. coli.*

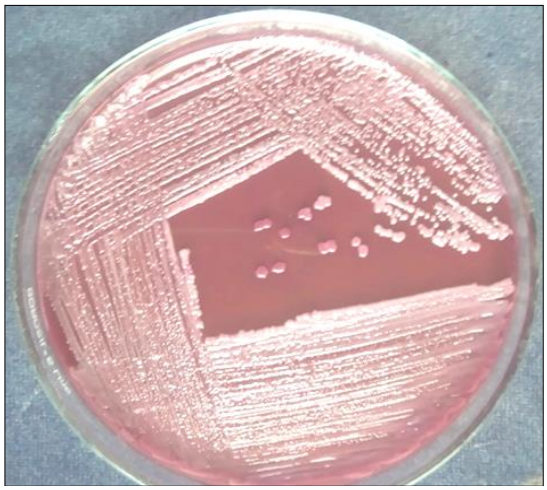


Photo plate 1: Colonies of *E. coli* on EMB Agar

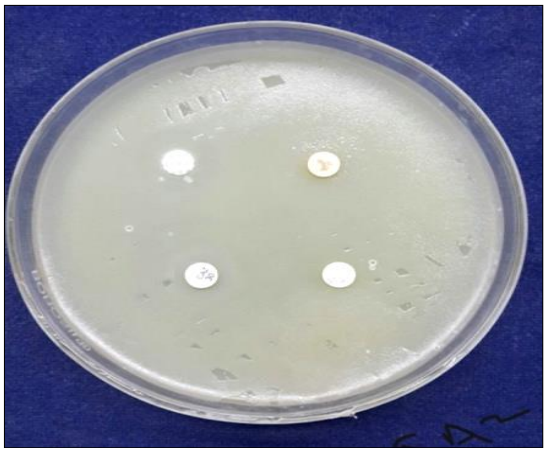


Photo plate 2: *E. coli* showing resistance to IE, CAZ, CTX and PEN.

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AGATGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGTAACAGGAAGCA
GCTTGCTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTG
ATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACC
AAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTA
GTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGA
CCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG
AATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGC
CTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGATGAGGTTAATAACCTCAT
CGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGT
AATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCG
GTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACT
GGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCG
TAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGC
TCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG
TAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCG
TTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACG
GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCT
TACCTACTCTTGACATCCAGAGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGAACT
CTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGT
CCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAA
GGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGC
CCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCT
CGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAAC
TCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAAT
ACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAG
AAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGG
TGAAGTCGTACAG

Fig 1: 16 S rRNA Sequence of *E.coli* Isolate.

Table 2: Sequences Producing Significant Alignments of *E. coli* Isolate

Accession	Description	Total Score	Query coverage	E value	Max Ident.
LC0564771	*Escherichia coli* plasmid pV266-a DNA,contig: V266-a_scaffold_6, strain: V266	2565	99%	0.0	99%

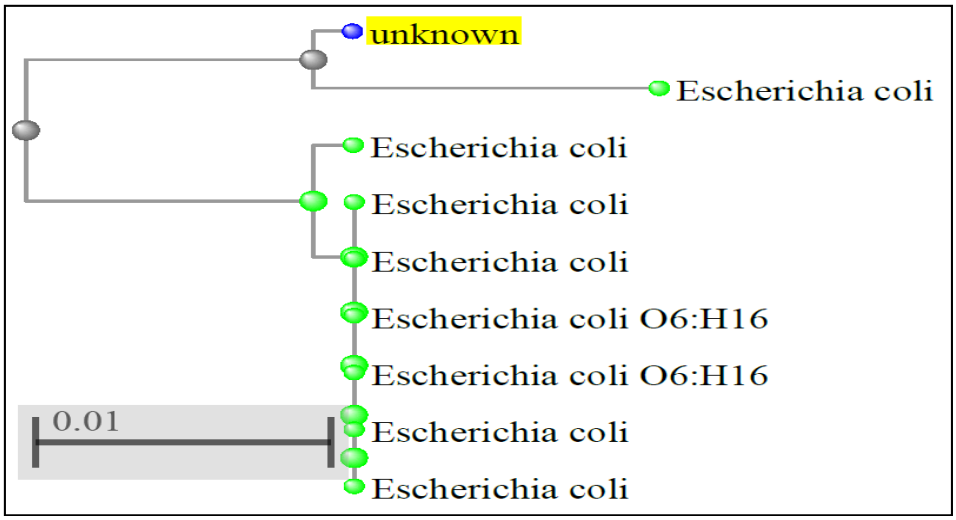

Fig 2: Phylogenetic tree of *E. coli* Isolate

Conclusion

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. It is concluded multi-drug resistant *E. coli* has emerged as the predominant pathogen responsible for early-onset neonatal sepsis, particularly in preterm infants.

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