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NEONATAL SEPSIS DUE TO COAGULASE-NEGATIVE (CONS) STAPHYLOCOCCI: FREQUENCY, ANTIBIOTIC SUSCEPTIBILITY AND MOLECULAR CHARACTERIZATION BY 16S RNA ANALYSIS

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ABSTRACT

Sepsis is a significant cause of morbidity and mortality in neonates. Hence, the present study was undertaken to identify the frequent organism and to detect antibiotic sensitivity pattern against the isolated organism in respect to better treatment. In present investigation, Out of 1000 neonates who admitted to preterm unit and intensive care unit (ICU) from different hospitals in Akola city, 104(14.1%) neonates were blood culture positive. *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.

KEYWORDS: Neonatal Septicemia, *Staphylococcus (CONS),* Antibiotic sensitivity, 16s rRNA analysis.

INTRODUCTION

Neonatal sepsis is a systemic inflammatory response caused by bacterial production of toxins after gaining entry into the bloodstream and multiplying.[3] It 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.[15],[16] Generally, it is a life-threatening case. In pre term babies, this can cross the blood-brain barrier since the baby is not fully developed, which might result in increased morbidity and mortality globally.[3,15] This demands immediate empirical antibiotic treatment after collection of blood culture samples.[9]

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.[19] 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.[6,11] Consequently, infants admitted to a hospital obtain most of their microorganisms from the hospital environment, their parents, and staff.[13]

Neonatal septicemia is one of the leading causes of neonatal mortality and morbidity worldwide. Hence, the present study was undertaken to isolate the bacteria, Coagulase-negative staphylococci causing neonatal sepsis and determine their antibiotic susceptibility pattern of Coagulase-negative *staphylococci* (CONS) isolates from blood cultures of neonates at a neonatal care unit in Akola city. Antibiotic resistance in skin-residing strains has been found to be low at birth but to increase rapidly during the first week of hospitalization.[7]

MATERIALS AND METHODS

The present study is based on a prospective analysis of 104 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.

BLOOD SAMPLES COLLECTION AND PROCESSING

Using aseptic conditions, 2 ml. blood was drawn and inoculated into brain‑heart infusion broth and incubated at 37°C. Subcultures were made on nutrient agar, blood agar, and MacConkey's agar. The inoculated plates were incubated under aerobic conditions for 24 hr. For confirmation again subcultures were made on selective

and specific media. Isolates were identified by std. Microbiological techniques.

Simultaneously, by using Kirby-Bauer disc diffusion method (1966) according to Clinical and Laboratory Standards Institute guidelines (CLSI).[2] Antibiotic susceptibility patterns of the coagulase negative *Staphylococcus (CONS)* 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. 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).

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. 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, Graph 2. Frequency of *Staphylococcus epidermidis* (CONS) was 104 (14.1%) (Table 2).

High rates of (CONS) infections were reported in the Middle East, Southeast Asia, and Latin America.[19] *Staphylococcus epidermidis* (CONS) has been identified as the causative organism for EOS as proved in other studies.[5,17,18] 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.[4,16] The increasing prevalence of (CONS) infections is attributable to their increasing antibiotic resistance as reported by earlier researchers.[14]

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.[13] *S.epidermidis(CONS)* prevalent isolates amongst the gram positive organisms showed high rate of antibiotic sensitivity to Imipenem, Meropenem as reported by investigators.[15] 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.[14]

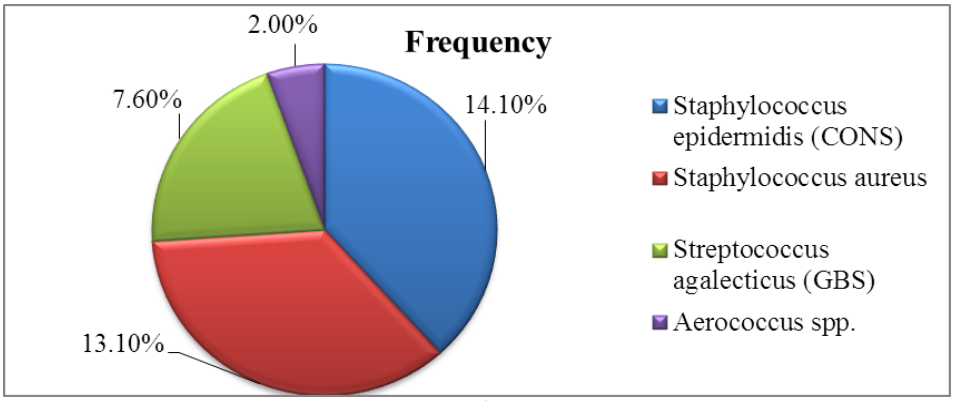
Table 1: Bacteriological Profile and Percent Distribution of Gram Positive Isolates Causing EOS and LOS.

Gm +ve Organisms	Early onset Sepsis (EOS)		Late onset Sepsis (LOS)	
	N	%	N	%
***Staphylococcus epidermidis* (CONS)**	49	23.67%	55	84.61%
Staphylococcus aureus	77	37.19%	20	30.76%
***Streptococcus agalecticus* (GBS)**	47	22.70%	9	13.84%
***Aerococcus* spp.**	9	4.34%	6	9.23%
Total	**207**	**76.2%**	**65**	**23.8%**

Table 2: Frequency Distribution of *Staphylococcus epidermidis* (CONS).

Gm +ve Organisms	Frequency (n)	Percent
***Staphylococcus epidermidis* (CONS)**	104	14.1%

In present investigation, high resistance of all isolates of *S. epidermidis(CONS)* was observed against Ampicillin as observed in the previous study[11] and Penicillin comparable to results reported in the study.[14]



Graph 1: Frequency Distribution of Gram Positive Bacterial Isolates.

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 3,Graph 03 and Photoplate 2). The increasing trend in the prevalence of MR-(CONS) has also been reported in other studies.[1,8,13] This is because of the indiscriminate use of antibiotics(Table 3, Graph 03).Gram positive isolates as well as the Gram negative isolates were highly sensitive to Imipenem (97.55%) and Meropenem (92.00%).

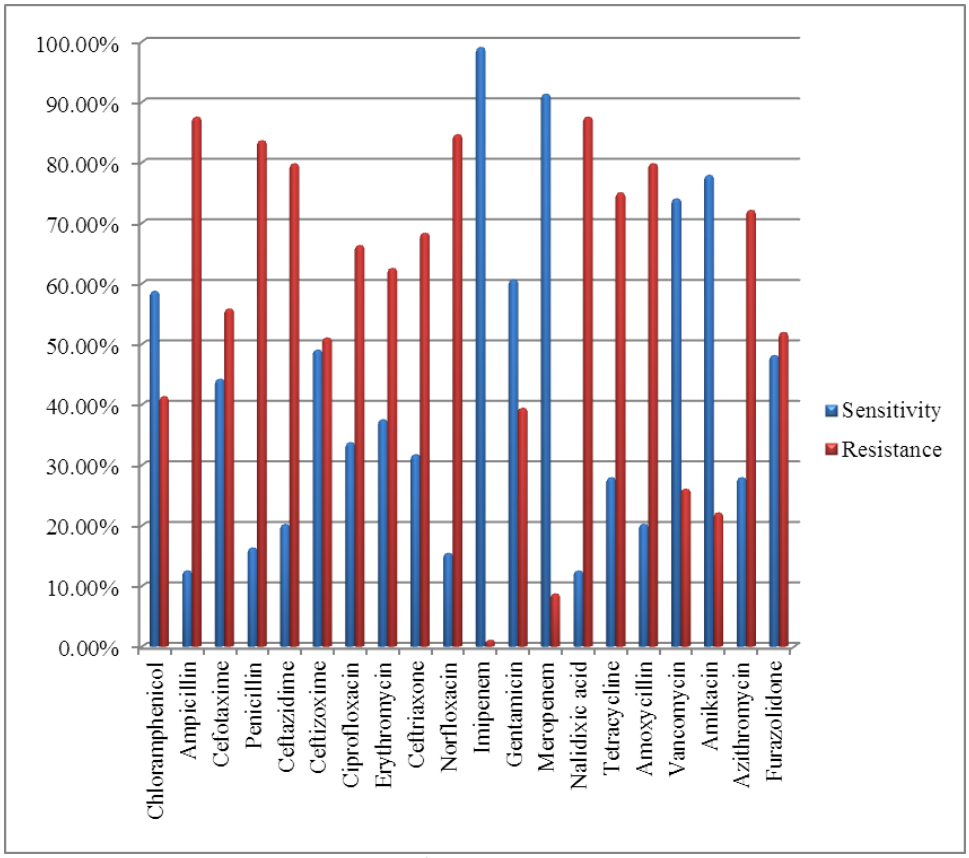
The primary identification of obtained isolates of ***Staphylococcus epidermidis*** **(CONS)** obtained was carried out based on cultural and biochemical characteristics (Photoplate1). Alignment Search Tool (BLAST) data base of National Center for Biotechnology (NCBI).[1] Information was used to compare 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. The revealed sequence accession number is NC 004461.1.NCBI, provided us taxonomy report with 74% similarity in BLAST analysis (Fig. 01, 02 and Table 04).

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. Molecular characterization by 16s rRNA analysis confirmed that neonatal septicemia is commonly caused by *Staphylococcus epidermidis* (CONS).

Table 3: 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%



Graph 03: Antibiogram of *Staphylococcus epidermidis*(CONS).

Table 04: Sequences producing significant alignments of *Staphylococcus epidermidis*(CONS) Isolate.

Accession No.	Description	Total Score	Query Coverage	E value	Max Ident.
NC_004461.1	Staphylococcus epidermidis ATCC 12228 chromosome, complete Genome	1993	82%	2e-109	74%

>15

CTATGGGTGGGGCGTGCTATAATGCAGTCGAGCGGACAGATGGGGCTTGCTCCCTTTTGTGTTCGCGGCA
CAGGGGAGAGACACGTGGGTAACCCCTCTGTATGACTGTGGTATCTCTTCGAAAACCGAGCTAATACCGG
ATATAACCTTGAACCCCGTGGGTCTCGGTTGAAAAAGGGTTTTTGCTGGCACTTTCTAATGGGCCCCCCGC
CCATTTACTTATTGGGGAGGGAACCGGTTTCCCAGGGGACCATTCGTAACCCAACTGGAAGGGGGGTCGG
GCCCCCTGGGACTGAAAACCGGGCCCAAATCCCTCCGGGGGGAACCATAGGGAAATTTCCCCAATGGGG
GAAAAGCTGACGGAACAACCCCCCCGGGGTGATGAAAGGTTTTCGAATCTAAAAATTTGGTGTTAGGGA
AAAAACAATGGTGGAATAACTGGTTGCACCTTTGCCGGACCCTACCCAAAAGCCCCCGGTAAATACCTTG
CCACAGCCGCGGTAATACCTTAGTGGGCAGCGTTGTCCCGAATTTTGGGCGTAAAGGCGCGCAGGGCGGT
TTTTAGTCTGAATGTGAAGGCCCCGCTCCACCGGGAGGGCCATGGAAATGGGAACTTGAATGAAAAAAG
AAGTGGAATCCAGGGTGCGGTGAATGCCTAGAGTTTGGAGGACCCCAGTGCGAGGCGCTCTCCGGTCTGT
AACTGACGCTGATAGCGAAGCTGGGAATGACAGAATAGATACCCTGGTAGTCCACGCCGTAACGATGAT
GCTAGTGTAAGGGTCGGCCCTAATGCTGCAGCTAACGCATAGCACTCGCTGGGATACGATCGCAGCTGAC
TCAGATGACGGATCGCAGCGTGACATGGTATCGAGCACGGAGACTACAGCTGAATCTGACGCTAGATAG
CTTCTGGACAGCATGGTCTGTTGCACTGTCGATGTATCCAACGGACGTACATGCCATTGTACTAGTATCGT
ACGAGAGTGTAGCATCA

Figure 01: 16 S rRNA Sequence of *Staphylococcus epidermidis*(CONS) Isolate.

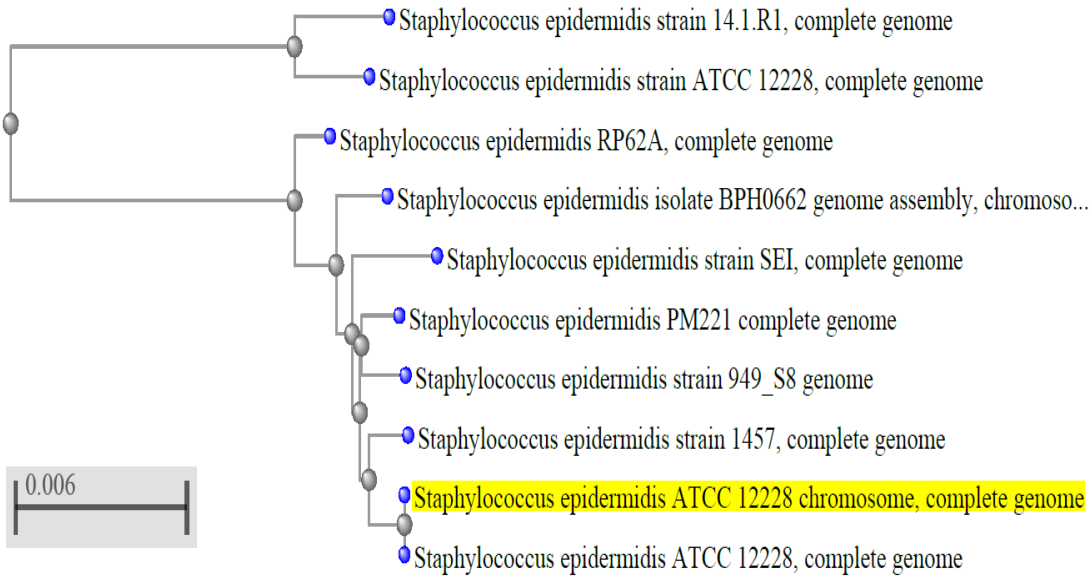


Figure 02: Phylogenetic tree of *Staphylococcus epidermidis*(CONS) Isolate.

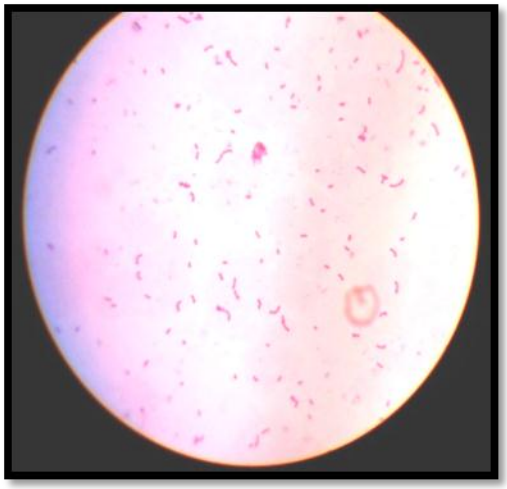


Photo plate 1: Microscopic image of (CONS).

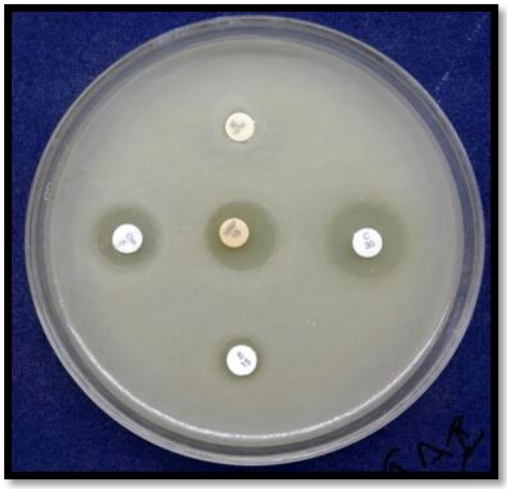


Photo plate 2: *Staphylococcus epidermis* showing susceptibility to C, CIP, E and MRP.

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