



CHARACTERIZATION OF COAGULASE NEGATIVE STAPHYLOCOCCI ISOLATED FROM NEONATAL SEPTICEMIAS IN A TERTIARY CARE CENTER.

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ABSTRACT

Background: Sepsis is the most common cause of death in neonates worldwide. Coagulase- negative staphylococci (CoNS) are now the leading cause of nosocomial bacteremia in most pediatric hospitals.

This study is done to speciate various CoNS isolated from neonatal sepsis cases and determine their antibiotic sensitivity profile. **Methods:** 75 cases of neonatal sepsis with CoNS bacteremia (from single BacT/ALERT PF bottle) formed the study group. The clinical isolates of CoNS were identified and their antibiograms determined by Vitek 2 system. MRCoNS were confirmed by *mecA* gene detection using conventional Uniplex PCR. **Results:** Among the CoNS isolates *S. epidermidis* accounted for 28% followed by *S. hominis* 27%, and *S. lentus* (26%), *S. haemolyticus* (11%), *S. saprophyticus* (6%), *S. warneri* (2%). All the isolates were sensitive to vancomycin & linezolid (100%). Followed by tetracycline 81%, cotrimoxazole 80%, gentamycin 64%. 79% of the isolates were methicillin resistant and had *mecA* gene. **Conclusion:** The study concludes that, all the CoNS isolated from neonatal sepsis may be speciated and sensitivity patterns determined for better management of cases. Emergence Methicillin resistant Coagulase negative staphylococci is an important nosocomial pathogens mainly in neonatal intensive care units was observed in this study.

KEYWORDS : CoNS, Neonatal sepsis, Methicillin, Antibigram, NICU.

INTRODUCTION

Sepsis is defined as a systemic inflammatory response of the host to an infection. Neonates are particularly vulnerable to sepsis because of weak immunological barrier and it is a major cause of mortality and morbidity worldwide¹. Neonatal sepsis can be categorized into early-onset sepsis (EOS) and late-onset sepsis, depending on the time of onset of infection. In early-onset neonatal sepsis, infections occur within 72 hours after delivery, while late-onset sepsis (LOS) occurs beyond 72 hours postpartum^{2,3}. Sepsis accounts for about 6 million neonatal and childhood deaths a year, accounting for 60-80% of annual child mortality⁴.

The pattern of organisms causing neonatal sepsis has been constantly changing and indiscriminate use of antibiotics has resulted in the emergence of multidrug resistant and virulent organisms.⁵ Prior to 1980, most neonatal septicemias were caused by gram negative bacilli and staphylococcus aureus.⁶

In the last two decades, however, Coagulase negative staphylococci (CoNS) has been emerging as the predominant causative organism⁷, which are often resistant to Methicillin.^{8,9}

In view of the small size of the patients and inaccessible vasculature in neonates, it is often not feasible to obtain more than one blood culture which is necessary for establishing the diagnosis of bacteremia, line colonization, or contamination. In this setting there is a need to identify all the CoNS isolates to the species level, their resistance pattern and to determine their significance as etiological agents to allow the judicious use of glycopeptide antibiotics.¹

OBJECTIVES

1. To speciate and determine the antibiograms of CoNS isolated from blood cultures of neonates with sepsis.

2. Detection of *mecA* gene in Methicillin resistant species by conventional PCR.

MATERIALS AND METHODS

Study period:

This study was conducted over a period of one year from July 2018 to June 2019.

Study design: A Validity study.

Inclusion criteria:

All the neonates admitted in the NICU of a tertiary care centre with presumptive evidence of sepsis with their blood cultures yielding CoNS.

Exclusion criteria:

Neonates with blood cultures yielding organisms other than CoNS.

Sample size: 75 cases of neonatal sepsis with CoNS bacteremia.

Methodology:

BacT/ALERT blood cultures (single culture) yielding CoNS received from NICU of a tertiary care center were further processed as per standard protocols. In all the neonatal sepsis cases about 1-2 ml of the blood is collected aseptically and added to BacT/ALERT PF (Ref. 410853) culture bottle and loaded in continuous monitoring BacT/ALERT 3D (BioMérieux) automated blood culture system and further processed as a routine protocol in our hospital. All the positively flagged blood cultures with gram positive cocci were subcultured on to Blood agar plates. Colonies suggestive of Staphylococci (white, opaque haemolytic or non haemolytic) were subjected to Gram staining, Catalase, slide

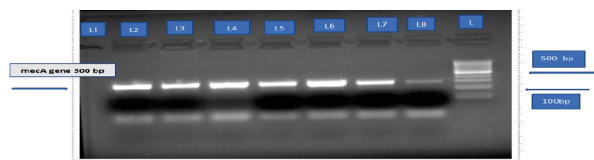
and tube coagulase tests were performed to confirm the identification of Cons. All the CoNS isolates were speciated and AST was performed in Vitek 2 systems (Biomeriux) by using GP ID (P 628) & GP AST cards .MRCoNS isolates were further confirmed for the presence of mecA gene by Conventional Uniplex PCR.

Primer sequence designed for mecA gene¹⁰

Primer	Sequence(5'-3')	Remark	Product size
MecA F	TGGCTATCGTGTACAA ATCG	Methicillin resistance	300bp
MecAR	CTGGAACCTGTTGAGC AGAG		

The PCR cycling protocol was applied as follows¹⁰

Initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 1 min, followed by a final extension at 72°C for 7 min. Agarose gel electrophoresis analysis of PCR amplification products was done and gel doc image captured.



Agarose gel electrophoresis analysis of PCR amplification products of mecA gene of 300 bp, extracted from CoNS isolates.

PCR products for mecA gene for positive MRCoNS isolates. Lane1: negative control (no DNA template); lane 2: positive control (mecA positive) Lane L: DNA molecular size marker (100 bp ladder)

The demographic and clinical details were reviewed from the case files

Statistical analysis:

MS EXCEL & EPI INFO 7 version statistical software package was used for statistical analysis.

Ethical consideration:

The study was approved by Ethical Committee of Gandhi Medical College, Secunderabad. The consent was taken from each subject included in the study.

RESULTS

Gender wise distribution of cases. Among the 75 positive CoNS blood cultures received 34(45%) were Females and 41(55%) were Males. Male to female ratio was 1.2:1.

Clinical profile of the cases Most of the neonates with septicemia presented with fever, difficulty in breathing, tachycardia, malaise, refusal of feeds or lethargy.

Table 1. Showing the clinical profile of the cases.

Clinical features	N %
Refusal of feeds	51 %
Vomiting	25 %
Abdominal distension	14 %
Jaundice	32 %
Respiratory distress	40 %
Lethargy	40 %
Fever	70 %
Tachycardia	53 %
pustules	4 %
cyanosis	5 %

Table 2. Showing distribution of cases in neonates according to onset of sepsis

Onset of sepsis	N (%)
Early onset sepsis	15 (20%)
Late onset sepsis	60 (80%)

In this study 80% of cases presented with late onset sepsis.

Spectrum of CoNS isolates

Among the CoNS species isolated *S. epidermidis* 28 % is the most common species followed by *S.hominis* 27%, *S.lentus* 26%, *S.haemolyticus* 11%, *S.saprophyticus* 6% and *S.warneri* 2%.

Table 3. Showing the spectrum of CoNS isolates

CONS SPECIES ISOLATED	NUMBER (N%)
<i>S.epidermidis</i>	21 (28%)
<i>S.hominis</i>	20(27%)
<i>S.lentus</i>	19(26%)
<i>S.haemolyticus</i>	9 (11%)
<i>S.saprophyticus</i>	5 (6%)
<i>S.warneri</i>	1 (2%)

Table 4. Risk factors in the study group.

Risk factor	N (%) 75
Low birth weight.	54 (72%)
Prematurity.	52 (70%)
Indwelling catheters.	63 (84%)
Prolonged hospital stay.	46 (62%)

Table 5. Showing species wise distribution of risk factors.

CONS SPECIES ISOLATED	NUMBER N%	Low birth weight N%	Prematurity. N%	Indwelling catheters. N%	Prolonged hospital stay N%
<i>S.epidermidis</i>	21 (28%)	18 (85%)	19 (90%)	21 (100%)	12 (57%)
<i>S.hominis</i>	20(27%)	14 (70%)	10 (50%)	13 (65%)	7 (35%)
<i>S.lentus</i>	19(26%)	12 (63%)	11 (58%)	13 (68%)	7 (36%)
<i>S.haemolyticus</i>	9 (11%)	7 (77%)	8 (89%)	9 (100%)	6 (66%)
<i>S.saprophyticus</i>	5 (6%)	3 (60%)	4 (80%)	5 (100%)	4 (80%)
<i>S.warneri</i>	1 (2%)	1 (100%)	-	1 (100%)	-

Presence of indwelling catheters was the most common risk factor (84%) followed by low birth weight 72% and prematurity (70%)

Antibiograms of the CoNS isolates.

Table 6. Distribution of methicillin resistance among CoNS species

CONS species ISOLATED	Number	MRCoNS. (Cefoxitin screen positive) N%
<i>S epidermidis</i>	21 (28%)	19 (89%)
<i>S hominis</i>	20 (27%)	14 (70%)
<i>S lentus</i>	19 (26%)	16 (84%)
<i>S haemolyticus</i>	9 (11%)	6 (72%)
<i>S saprophyticus</i>	5 (6%)	3(66%)
<i>S warneri</i>	1 (2%)	1 (50%)
Total	75	59 (79%)

We observed 79% of the isolates were resistant to Methicillin and were confirmed for the presence of mecA gene.

Table 5. Sensitivity pattern of MRCoNS and MSCoNS to other Antibiotics.

Antibiotic	MRCo NS (N=59)				MSCoNS (N= 16)			
	R(N)	R(%)	S(N)	S (%)	R(N)	R (%)	S(N)	S(%)
Erythromycin	48	82%	11	12%	1	7%	15	93%
levofloxacin	46	78%	13	22%	4	28%	12	72%

Ciprofloxacin	46	78%	13	22%	5	31%	11	69%
Clindamycin	31	52%	28	48%	1	7%	15	93%
Gentamicin	15	26%	44	64%	7	46%	9	54%
Cotrimoxazole	12	20%	47	80%	1	7%	15	93%
Tetracycline	11	19%	48	81%	1	7%	15	93%
Vancomycin	0	0%	59	100%	0	0%	16	100%
Linezolid	0	0%	59	100%	0	0%	16	100%

High resistance to methicillin was observed among all the species. In MRCoNS isolates percentage of resistance was higher for quinolones and erythromycin. All the isolates were sensitive to vancomycin and linezolid. Emergence of new species of CoNS (23%) of *S.lentus* as pathogen can be observed from this study.

DISCUSSION

Spectrum of CoNS species isolated

Author	Claus Klingenberg 11	G. Dimitriou et al 12	Mohd. Mubashir Ahmad Khan et al 13	Dr B. Senthilselvan 14	Nazir A. et al 15	Present study
Year	2005	2011	2014	2019	2019	2019
Place	Norway	Greece	Makkah Saudi Arabia	Thanjavuru Tamilnadu	Kashmir	Telangana
<i>S. epidermidis</i>	72%	59.4%	75.85%	53%	17%	28%
<i>S. hominis</i>	5%	5.9%	9.4%	-	-	27%
<i>S. lentus</i>	-	-	-	-	2%	26%
<i>S. hemolyticus</i>	11%	31.8%	11.1%	41%	44.6%	11%
<i>S. saprophyticus</i>	-	0.6%	-	-	-	6%
<i>S. warneri</i>	9.5%	1.2%	1.1%	3%	14%	2%
<i>S. capitis</i>	2.2%	-	0.5%	-	-	-
<i>S. schleferi</i>	-	-	-	3%	2%	-
<i>S. lugdunensis</i>	-	1.2%	0.5%	-	3%	-
<i>S. xylosus</i>	-	-	-	-	3%	-

The results of our study are similar to those done by Mohd.Mubashir Ahmad Khan et al, Makkah who reported 75.8% *S epidermidis* followed by Claus Klingenberg, Norway 72% *G. Dimitriou et al Greece* 59.4% ,and Dr B. Senthilselvan et al 53% who reported highest percentage of *S epidermidis* & Followed by other species. We reported 26% of *S.lentus* which is distinct from other studies.

Various studies on percentage of MRCoNS

Author	Year	Place	%MRCoNS
Mohd. Mubashir Ahmad Khan et al 13	2014	Makkah Saudi Arabia	93.6%
Nazir A. et al 15	2019	Kashmir	84%
Dr B. Senthilselvan 14	2019	Thanjavur	76%
Present study	2019	Telangana	79%

The results of the present study reported 79% of MRCoNS. Which is similar to a study by Dr B. Senthilselvan et al who showed 76% MRCoNS.

Other studies by Mohd. Mubashir Ahmad Khan et al, Nazir A. et al showed 93.6% and 76% of MRCoNS respectively

Various studies on the resistance pattern of other antibiotics in MRCoNS include

YEAR	2014	2019	2019	2019
Author	Mohd. Mubashir Ahmad Khan et al,13	Nazir A. et al 15	Dr B. Senthilselvan 14	Present study
Place	Saudi Arabia	Kashmir	Thanjavuru Tamilnadu..	Telangana
Antibiotic	-	-	-	-
Erythromycin	78%	-	74%	82%
Cotrimoxazole	-	-	74%	20%
Ciprofloxacin	-	47%	-	78%
Gentamicin	82%	35%	-	26%
Clindamycin	60%	44%	76%	52%
Vancomycin	0%	0%	0%	0%
Linezolid	0%	0%	0%	0%
levofloxacin	-	-	-	79%
Ampicillin	99%	-	82%	-

The present study showed similar pattern of drug resistance as that of Mohd. Mubashir Ahmad Khan et al and Dr B. Senthilselvan et al.

CONCLUSION

Methicillin resistant Coagulase negative staphylococci is still an important nosocomial pathogen in NICUs and is a also source of resistance genes to other gram-positive cocci including *Staphylococcus aureus*. Emergence of methicillin resistant species of *S.hominis* (26%) and *S.lentus*(23%) was observed in our hospital settings. Methicillin resistance in CoNS is associated with a pattern of multidrug resistance in their antibiograms. Speciation of CoNS and determining their antibiogram is useful in better management of neonatal sepsis.

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