

## Original Research Article

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## Multi Drug Resistant *Staphylococcus haemolyticus* An Emerging Nosocomial Pathogen in Neonatal Sepsis at Tertiary Care Centre, Thanjavur, India

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**A B S T R A C T**

*Staphylococcus haemolyticus* is part of the human normal skin microflora and is also found in nonhuman primates. This organism has been documented as a cause of primary and nosocomial bacteremia in clinical health care settings. Though CONS is ruled out as a contaminant, the emergence of multidrug-resistant isolates to glycopeptides, methicillin, fluoroquinolones, macrolides, lincosamides, streptogramin B and linezolid results in treatment failure in primary bacteremia. Transmission of resistant clones among *Staphylococcus* species necessitates the correct identification of *Staphylococcus* species for prompt treatment and identification plays a vital role in the prevention of nosocomial spread among healthcare workers. In this retrospective study we identified *Staphylococcus haemolyticus* from blood cultures sent to microbiology department from neonatal intensive care unit during the period from January to December 2018 by antimicrobial susceptibility testing and tested for various resistance patterns by CLSI standards. This study signifies the importance of identification of *Staphylococcus haemolyticus* species among CONS in neonatal sepsis and it's various resistance pattern to increase the antibiotic stewardship in clinical healthcare settings.

**Keywords**

*Staphylococcus haemolyticus*,  
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**Introduction**

*Staphylococcus haemolyticus* is a coagulase-negative member of the genus *Staphylococcus*. The bacteria can be found on normal human skin flora and can be isolated from axillae, perineum, inguinal areas of humans. It is the second most isolated CONS presenting in human blood. Coagulase-negative staphylococci are considered low virulent pathogens comparing to the well known pathogenic coagulase positive *Staphylococcus aureus*. *Staphylococcus haemolyticus* is a

remarkable opportunistic pathogen well known for its higher antibiotic resistant phenotype. They cause meningitis, skin and soft tissue infections, prosthetic joint infections, and bacteremia. The ability of *Staphylococcus haemolyticus* to simultaneously resist various antibiotics has been observed and studied for a long time. The resistance genes for each type of antibiotic can be located on the chromosome (Methicillin), on the plasmids (macrolides), or on both chromosome and plasmids (aminoglycosides) along with their ability to

produce beta-lactamases and its ability to alter penicillin-binding protein by expressing *mecA* gene. The present study aimed to understand the antibiotic susceptibility pattern of *Staphylococcus haemolyticus* to prevent therapeutic failure by these multidrug-resistant strains.

## Materials and Methods

### Clinical Isolates

Blood cultures sent to the microbiology department in suspected cases of neonatal sepsis in a tertiary care center at Thanjavur from January 2018 to December 2018 and were screened for various resistance patterns of *Staphylococcus haemolyticus* species. Out of 835 samples received, 96 blood cultures were positive for coagulase-negative *Staphylococcus* species out of which 39 belonged to *Staphylococcus haemolyticus* identified by various phenotypic detection methods. we isolated the same with the repeat blood cultures collected from neonates under strict aseptic precautions diagnosed with bacteremia. The above isolates were subjected to antimicrobial susceptibility testing using standard disc diffusion procedure and tested for various resistance patterns according to CLSI guidelines.

### *Staphylococcus haemolyticus* species identification

Catalase positive; Slide and tube coagulase negative; Blood agar shows beta hemolysis Novobiocin sensitive; Ornithine not decarboxylated; Urease negative; Mannose not fermented; Acetoin produced (*S.haemolyticus*).

### Antimicrobial susceptibility profile

Antimicrobial susceptibility of *S.haemolyticus* strains were performed on Mueller Hinton agar by Kirby Bauer disc diffusion method to

demonstrate beta-lactamase production by penicillin zone edge test, cefoxitin disk screen test(MR CONS), D test (iMLSb), vancomycin, linezolid, and cefoxitin EZY MIC strip and the results were interpreted according to CLSI guidelines and resistance to linezolid was confirmed by PCR.

### Penicillin zone edge test

Standard disk diffusion procedure, penicillin 10U disk is placed in Mueller-Hinton agar plate after inoculating the test strain and the same incubated at 37°C for 16-18 hours. sharp zone/cliff edge demonstrates beta-lactamase production. fuzzy zone/ beach edge is beta-lactamase negative, halo with a diameter less than or equal to 28 mm is resistant.

### Cefoxitin disk screen test

Cefoxitin is a second generation cephamycin antibiotic that induces the expression of *mecA* gene that codes for the altered penicillin-binding protein (pbp2a). Cefoxitin is used as a surrogate marker for methicillin resistance by CLSI. for the test standard disk diffusion procedure, cefoxitin 30 µg disk placed in Mueller Hinton agar plate incubated at 37°C for 16-18 hours and zone of inhibition less than 24 mm is considered as methicillin-resistant for staph haemolyticus.

### D test

A disk of 2 µg of clindamycin was placed at a distance of 15-20 mm from the edge of the disk of 15µg of erythromycin in MH agar plate and after incubation at 37°C for 16-18 hours, isolates that shows no flattening of the inhibition are susceptible to clindamycin (negative D test) and isolates that shows flattening of the inhibition zone around the clindamycin disc indicates inducible clindamycin resistance (positive D test) confirms the presence of *ermA* gene modification.

## Linezolid resistance

Linezolid resistance identified by modified Kirby Bauer disk diffusion method was performed using 30µg linezolid disk on Mueller Hinton agar as per CLSI standards and *cfr* (chloramphenicol-florfenicol resistance) gene responsible for linezolid resistance was identified by using PCR.

## Results and Discussion

Out of the 835 (n) isolates, Gram-negative bacteremia accounted for 37%(n=314) of neonatal sepsis, Gram-positive species accounted for 20%(n=170) of neonatal sepsis and candida accounted for 1% (n=12) of neonatal sepsis. Among the gram-positive cocci MR CoNS (11.5%, n = 96) was more prevalent than MRSA (5.7%) and *Enterococcus* (3.1%).

96 species of MR CoNS were identified of which 39 (4.6%) isolates were identified as *Staphylococcus haemolyticus*, All these strains were methicillin resistant (100%), Penicillin disc zone edge test revealed sharp zone/cliff edge demonstrating beta-lactamase production(100%) and 4 species were iMLSb phenotype [ERY (R) + CD (S) positive D test ] and 35 species were cMLSb phenotype [ERY(R)+ CD (R) negative D test]. Two species were showing resistance to linezolid. All these strains were susceptible to vancomycin.

Traditionally, the production of coagulase is considered to represent the invasive pathogenic potential among staphylococci. *Staphylococcus haemolyticus* is a coagulase-negative species and emerged as a major cause of nosocomial infection especially in neonatal sepsis. All strains of *S. haemolyticus* produce hemolysins (substance that breaks down red blood cells) *invitro* and this hemolysin is considered to be responsible for high virulence of this species.

Comparative genomic analysis of various studies reveals significant similarities between the genomes of *Staphylococcus haemolyticus* with *S. aureus* and *S. epidermidis* the studies also found out that a region of the chromosome called as 'oric environ' is unique for the above organisms.

*S. haemolyticus* also possess a surprisingly large number of insertion sequences (ISs), these can either inactivate a gene by direct integration or activate a gene by the potential promoter. By changing the content of the genome the IS elements contribute to the innate ability of the bacteria to acquire drug resistance. With this ability *Staphylococcus haemolyticus* makes themselves a remarkable and hard to control opportunistic pathogen. Also, certain studies reveal strains of *Staphylococcus haemolyticus* only require arginine for growth, while *Staphylococcus aureus* requires availability of many different amino acids for their growth.

*S.haemolyticus* with its wide range of genetic diversity is a reservoir of various resistance genes and evidence has suggested that there is the potential possibility of horizontal transfer of sccmec type V from MR *Staphylococcus haemolyticus* to Methicillin-susceptible *Staphylococcus* species. Linezolid is a synthetic drug belonging to the oxazolidinone group so there will be no natural reservoir of resistance genes for developing resistance. The possible resistant mechanism is being the presence of point mutations at the drug target site.

The most frequent mutation is G2576T and this is not transmissible between staphylococcal species but a new mechanism of linezolid resistance involving the acquisition of a natural resistance gene, *cfr* (chloramphenicol-florfenicol resistance) is non-mutational and is transferred by means of horizontal transfer via plasmids among staphylococcal species (Fig. 1–7; Table 1 and 2).

**Table.1** CoNS speciation by the simple scheme

s.no	Species	Slide coagulase test	Tube coagulase test	Ornithine decarboxylase test	Urease activity	Mannose fermentation	Novobiocin sensitivity (5µg)
1	<i>S.epidermidis</i>	-	-	+	+	+	S
2	<i>S.saprophyticus</i>	-	-	-	+	-	R
3	<i>S.haemolyticus</i>	-	-	-	-	-	S
4	<i>S.lugdunensis</i>	+	-	+	+	+	S
5	<i>S.warneri</i>	-	-	-	+	-	S
6	<i>S.cohnii</i>	-	-	-	+	-	R

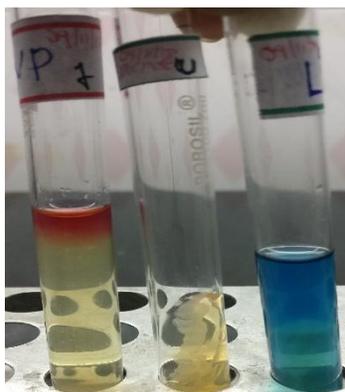
**Table.2** Antibiotic susceptibility pattern of *Staphylococcus haemolyticus*

s.no	Antibiotic susceptibility tests	Total number of resistant <i>S.haemolyticus</i> (n)	Percentage of resistance to antibiotics. (%)
1	Beta-lactamase production	39	100
2	<i>mecA</i> gene detection by cefoxitin disk test	39	100
3	cMLSb phenotype	35	89.7
4	iMLSb phenotype (erm gene) D test	4	10.3
5	Vancomycin resistance	Nil	Nil
6	Linezolid resistance (cfr gene)	2	0.5

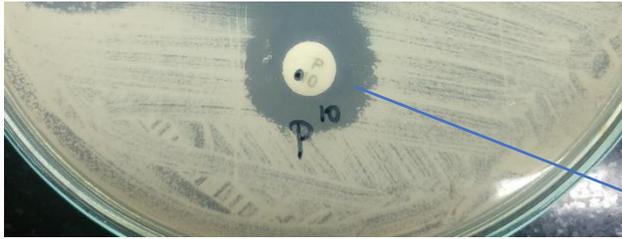
**Fig.1** *S.haemolyticus* on blood agar showing beta hemolytic white opaque colonies



**Fig.2** BCR of *S.haemolyticus* with urease negative and acetoin production

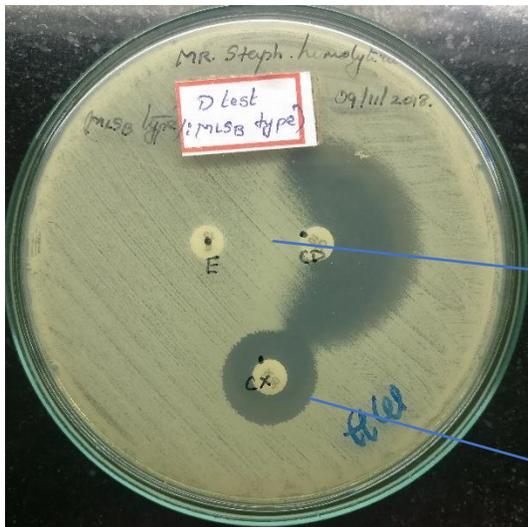


**Fig.3** Demonstrating penicillin zone edge test with sharp zone/cliff edge



sharp zone/cliff edge – beta-lactamase production positive

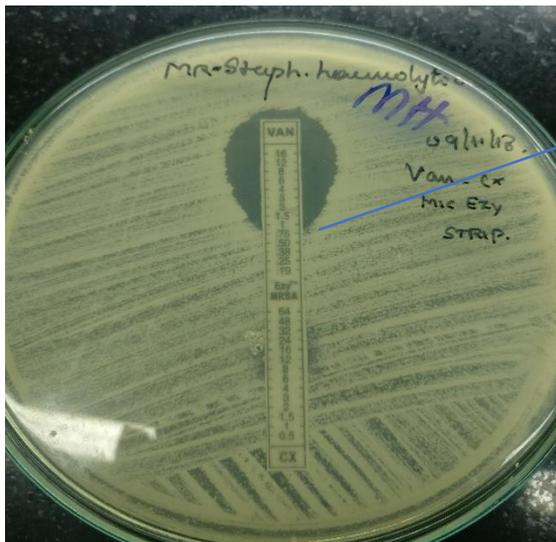
**Fig.4** Demonstrating both positive D test (iMLSb phenotype) and cefoxitin disk screen test (*mecA* gene detection)



Positive D test -flattening of the inhibition zone around the clindamycin disc adjacent to erythromycin disc (iMLSb type).

Cefoxitin zone of  $\leq 24$ mm indicates Methicillin resistance.

**Fig.5** Demonstrating vancomycin susceptible strain of *S.haemolyticus* with MIC of 1.0 mcg/ml

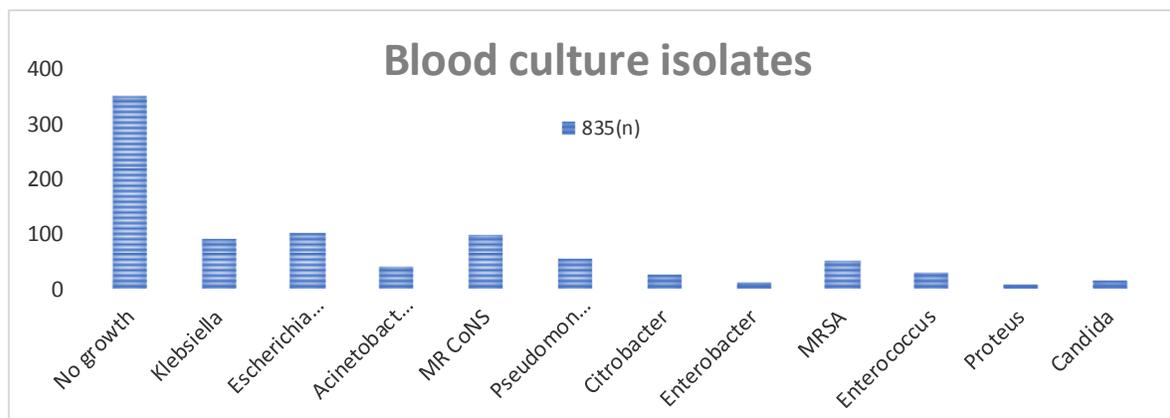


The MIC of 1.0 mcg/ml for vancomycin indicates that strain is vancomycin-susceptible.

**Fig.6** Gel electrophoresis band pattern positive for *cfr* gene



**Fig.7** Organisms isolated during the study and its statistical analysis



The product of the *cfr* gene is a methyltransferase that catalyzes methylation of A2503 in the 23S rRNA gene of the large ribosomal subunit, conferring resistance to chloramphenicol, florfenicol, and clindamycin.

In conclusion, *Staphylococcus haemolyticus* is an emerging superbug among *Staphylococcus* species because of its ability to produce hemolysins and its ability to resist various classes of antibiotics, This study emphasizes

the identification of *Staphylococcus haemolyticus* from other CONS in neonatal sepsis and its various resistance patterns similar to *Staphylococcus aureus* but surprisingly all these strains were susceptible to vancomycin. With the above, we need to speciate CONS before ruling out it as a contaminant and also we need to look for various resistance patterns, this will increase the antibiotic stewardship in clinical health care settings.

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