

## Staphylococcus Haemolyticus; a Nosocomial Pathogen Showing Higher Antimicrobial Resistance



### Medical Science

**KEYWORDS :** Coagulase Negative Staphylococci (CoNS), Staphylococcus haemolyticus, identification, Antibiotic susceptibility

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### ABSTRACT

*Coagulase Negative Staphylococci (CoNS) species Staphylococcus haemolyticus (S. haemolyticus) is 2nd or 3rd most common cause of nosocomial infection and showing multidrug resistance. An attempt was made and 199 CoNS isolates were collected from various clinical samples; blood cultures, pus, urine samples, vaginal swabs, miscellaneous samples and antibiogram of all isolates studied after speciation. 199 CoNS isolates with the help of practical scheme developed in our laboratory after going through various references. Scheme was able to identify 197 isolates up to species level. The S. haemolyticus is second most common species next to S. epidermidis and showing higher resistance to antibiotics. Few other species were also identified. The increase recognition of pathogenic potential of CoNS and emergence of drug resistance among them denotes the need to understand their antibiogram at their species level to plan empirical therapy.*

### INTRODUCTION

In the past Coagulase Negative Staphylococci (CoNS) were generally considered to be contaminants having little clinical significance [1]. However in recent time CoNS emerging as significant pathogens especially in medical devices related infection and in immunocompromised patients [2].

Many species have been described; relatively few are responsible for human diseases. CoNS species *Staphylococcus haemolyticus* (*S. haemolyticus*) is most prevalent species isolated from different clinical specimen next to *Staphylococcus epidermidis* (*S. epidermidis*) observed in many studies [3,4,5]. *S. haemolyticus* frequently colonizes the skin, mucous membranes of hospitalized patient [3] including neonates [6]. *S. haemolyticus* along with the *S. epidermidis* is well documented cause of blood stream infection in all ages [3,4], in neonates [6], wound infection, skin and soft tissue infection [7], infection in patient on different indwelling devices (Continuous ambulatory peritoneal dialysis catheter, thoracotomy tube, intraabdominal catheter/drain) [2,4], urinary tract infection [4]. Other species like *S. saprophyticus*, *S. hominis* etc also reported time to time [2,8].

Different study on antibiotic susceptibility pattern of CoNS strains were showing variability in sensitivity & resistance pattern [9, 10]. A large number of CoNS strain recovered from clinical specimen have become a serious problem as they are expressing methicilline resistance which involve  $\beta$  lactam antibiotic and lead to significant limitation of therapeutic options [4]. Additionally emerging strains of *S. haemolyticus* with decreased susceptibility to vancomycin, teicoplanin also has been reported [4]. The *S. haemolyticus* is highly prevalent in hospitalized environment with tendency to develop resistance to multiple antibiotic [4]. Considering CoNS Species are one of the most frequent cause of nosocomial infection and reservoir of multidrug resistance (MDR) especially *S. haemolyticus*, The present study aimed to understand the antibiotic susceptibility pattern of *Staphylococcus haemolyticus* and other CoNS isolates after their identification up to species level, to prevent major therapeutic problem in future by these MDR resistance strains and rationalized the use of these lifesaving antimicrobial weapons.

### MATERIAL AND METHODS:

A total 199 non repeated clinically significant CoNS isolates were collected in department of microbiology from clinical specimens include: Blood cultures, Pus samples, Urines, Vaginal swabs, and Miscellaneous samples (drain, catheters, fluids).

The strain collected were initially identified by colony morphology (sheep blood agar, nutrient agar), gram staining, catalase, coagulase (both slide and tube), for 4,24hrs and anaerobic acid from mannitol. All the strains which were either slide or tube coagulase negative further identified by scheme developed in our laboratory after reviewing number of references and test selected from Kloos and Schleifer scheme [1,2,8]. Test used were acetoin, urease production, ornithine decarboxylation,

glucose, lactose, mannose, trehalose, sucrose fermentation, Novobiocin, Bacitracin, and Furazolidone susceptibility. All test were performed according to reference method [1].

Antibiotic susceptibility of all CoNS strains were performed on Mueller Hinton agar by Kirby Bauer disc diffusion method as per CLSI guidelines against commonly used antibiotics; penicillin (10u), amoxicillin (20µg), erythromycin (15µg), ciprofloxacin (5µg), gentamicin (10µg), cefotaxime (30µg), cotrimaxazole (25µg), chloramphenicol (25µg), tetracycline (30µg), amikacin (30µg), vancomycin (30µg), methicillin (5µg) resistance was tested on Mueller Hinton agar supplemented with 5% sodium chloride and incubated at 35°C for 40hrs.

### RESULTS:

Out of 199 CoNS isolates; 92 were obtained from blood cultures, 38 from urine samples, 31 from pus, 23 from miscellaneous samples and 15 from vaginal swabs. Among species distribution *Staphylococcus haemolyticus* obtained as 2<sup>nd</sup> most common species 36/199 (18.0%) next to *S. epidermidis* 135/199 (67.8%), few other species were *Staphylococcus saprophyticus* 18/199 (14.7%), *S. lugdunensis* 3/199, *S. cohnii* 02/199, *S. schleiferi* 02/199, *S. hominis* 01/199 also identified. Two isolates were not able to identify because of aberrant reaction. The "Sources of *Staphylococcus haemolyticus* shown in table -1. However The *S. haemolyticus* is most common species found in miscellaneous group of samples 14/23 (60%).

**Table -1 "Sources of *Staphylococcus haemolyticus*"**

**TABLE - 1**

**SOURCES OF S. HAEMOLYTICUS ISOLATES (36)**

| sources               | No of isolates (%) |
|-----------------------|--------------------|
| Blood cultures        | 15(41.6)           |
| Pus                   | 04(11.1)           |
| Miscellaneous samples | 14(38)             |
| Vaginal swabs         | 02(5.0)            |
| Urine samples         | 01(2.5)            |
| Total                 | 36                 |

Maximum strains of *S. epidermidis* obtained from blood cultures, and *S. saprophyticus* from urine samples as their role already been established in urinary tract infection.

Antibiotic susceptibility pattern studied separately for *S. haemolyticus* and other species; revealed variability in resistance and sensitivity pattern. Result obtained in current study summarized in Table-2. The *S. haemolyticus* strains showing higher resistance to different antibiotics compare to other CoNS species and showing 75% resistance to methicilline more than other species.

**"Table-2 - Antibiogram of CoNS isolates"****TABLE - 2****ANTIBIOGRAM OF CoNS ISOLATES (n=199)****Proportion (%) of isolates sensitive(S) and resistance(R)**

| Antimicrobial agents |   | S.haemolyticus<br>n=36(%) | S. epidermidis<br>n=135(%) | Other CoNS<br>n=28(%) |
|----------------------|---|---------------------------|----------------------------|-----------------------|
| Penicillin           | S | 3 (8.3)                   | 14(10.3)                   | 3(10.7)               |
|                      | R | 33(91.7)                  | 121(89.7)                  | 25(89.95)             |
| Amoxicillin          | S | 4(11.1)                   | 19(14.0)                   | 5(17.8)               |
|                      | R | 32(88.9)                  | 116(86.0)                  | 23(82.2)              |
| Erythromycin         | S | 8(22.2)                   | 37(27.4)                   | 9(32.1)               |
|                      | R | 28(77.8)                  | 98(72.6)                   | 19(67.9)              |
| Chloramphenicol      | S | 16(44.4)                  | 65(48.1)                   | 15(53.5)              |
|                      | R | 20(55.5)                  | 70(51.9)                   | 13(46.4)              |
| Gentamicin           | S | 9(25)                     | 45(33.3)                   | 10(35.7)              |
|                      | R | 27(75)                    | 90(65.7)                   | 18(64.2)              |
| Cotrimaxazole        | S | 10(27.7)                  | 42(31.1)                   | 9(32.1)               |
|                      | R | 26(72.3)                  | 93(68.9)                   | 19(67.9)              |
| Ciprofloxacin        | S | 12(33.3)                  | 46(34.1)                   | 10(35.7)              |
|                      | R | 24(66.7)                  | 89(65.9)                   | 18(64.3)              |
| Cefotaxime           | S | 10(27.7)                  | 38(28.3)                   | 9(32.1)               |
|                      | R | 26(72.3)                  | 97(71.7)                   | 19(67.9)              |
| Tetracyclin          | S | 14(38.8)                  | 54(40)                     | 13(46.4)              |
|                      | R | 22(61.2)                  | 81(60)                     | 15(53.6)              |
| Amikacin             | S | 21(58.3)                  | 83(61.4)                   | 19(67.8)              |
|                      | R | 15(41.7)                  | 52(58.5)                   | 9(32.2)               |
| Methicilline         | S | 9(25)                     | 45(33.3)                   | 11(39.2)              |
|                      | R | 27(75.5)                  | 90(66.7)                   | 17(60.8)              |
| Vancomycin           | S | 36(100)                   | 135(100)                   | 28(100)               |
|                      | R | 00                        | 00                         | 00                    |

Figures in parentheses are the percentages

**DISCUSSION:**

Coagulase negative Staphylococcus are increasingly recognized as significant nosocomial pathogen[3,6]. Among species the *S. haemolyticus* is 2<sup>nd</sup> most documented cause of variety of infection [4,6] like prosthetic valve endocarditis, wound infection [7,11], bacteremia[3,4] etc. The references regarding study of antibiotic susceptibility pattern separately for species not much available in India ,may be because they are considered as normal inhabitant of skin nares[3]and the facility for CoNS species identification is not available in most laboratories and the process of identification of species by reference methods of Kloos and Schleifer cumbersome and time consuming ,available automated system still out of reach to most laboratories in developing country like India[1].

The species identification is important in monitoring reservoir and distribution of CoNS involved in nosocomial infection and determined the etiological agent [6] and antibiogram will be helpful in selecting the empirical therapy.

The present scheme developed in our lab after going through

references [1, 2, 8]. It include simple, feasible test that are able to discriminate between CoNS species. Scheme was able to identified one hundred ninety seven isolates up to species level. In present study maximum isolates were *S. epidermidis* 67.8% followed by *S. haemolyticus* 18%, correlate with studies [3, 4] but not with [8,9] may be because of different sample size ,distribution of species varied in different geographical region and diagnostic protocol used by different clinician. The *S. haemolyticus* is most frequent species obtained from miscellaneous samples (catheter, drains, fluids) 14/23(60%), more than other samples; favors the finding of other authors [2, 3] that it's common association with patients on indwelling devices.

Current study on antibiotic susceptibility pattern showed that *S. haemolyticus* have high resistance for many antibiotics penicillin(91.3%), amoxicillin (88.9%), erythromycin (77.8%), gentamicin(75%), cotrimaxazole(76.3%), cefotaxime(76.3%), correlate with study done by E.M. Barros et.al; penicillin(95%), gentamicin (73%) not correlate with other antibiotic susceptibility pattern. In current study resistance to erythromycin (77.8%) gentamicin (75%) similar to the J.W. Fogget et. al; erythromycin(79%) gentamicin(79%) and study done by another authors[6] showing (83.3%) resistance to erythromycin ,gentamicin both. Present study showing 75% resistance to methicilline ,bit lower than other author 88% [7], 80%[4], may be because of the varied geographical distribution of strain as well as discordant finding between phenotypic test and genotypic characterization of various strains of *S. haemolyticus* [7]. No strain of *S. haemolyticus* ,including all CoNS isolates showing resistance to vancomycin, correlate with other study [9,10]. Current study *S. haemolyticus* is showing higher antimicrobial MDR in comparison to other CoNS strains, individual variation, favors study[7] Present study showed variable antibiogram of *S. epidermidis* and other species, comparable to some study[9,10] for individual antibiotic not for others ,depicted in table- 2. Previous study done for antibiotic susceptibility pattern of all CoNS strains, [3] comparable to the antibiogram of *S. epidermidis* because this was the most frequent species found in most study 82.3%[9], 82.2%[3].

**CONCLUSIONS**

The *S. haemolyticus* is an important species of CoNS showing MDR, and associated with infection in patient on indwelling devices. In present study variable resistance /sensitivity shown by all CoNS isolates against individual antibiotic, none had predictable pattern of antibiogram. Therefore it is appear to be mandatory that individual isolate should be identified up to species level with their antibiogram as no particular pattern can be predicted in any problematic situation.

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