Original article:

**Study of prevalence of methicillin and vancomycin resistance in multidrug resistant coagulase negative staphylococci.**

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Abstract:

**Introduction:** Recently due to the improvements in the treatments of serious diseases, there are more and more patients whose resistance to infection is severely reduced. In recent years CoNS has emerged as a pathogen in growing number of serious nosocomial infections. Treatment of these infections caused by CONS, is increasingly problematic due to frequent occurrence of multiple antibiotic resistance. With the background in mind we planned the present work to study of prevalence of methicillin and vancomycin resistance in multidrug resistant Coagulase Negative Staphylococci.

**Material & Methods:** The present study was conducted in neonatal intensive care unit (NICU) of a tertiary care hospital over a period of one year. A Total of 972 neonates both preterm and fullterm, who were clinically suspected of having sepsis were included in the study. Blood, Pus, CSF and Urine specimen were collected. These samples were processed by standard bacteriological techniques. 269 samples from NICU environment were also collected during same time and period. Direct microscopy & blood culture was done. Antimicrobial susceptibility testing of the isolated strain was carried out according to modified Kirby Bauer disc diffusion method.

**Observations & Results:** Antimicrobial susceptibility testing of the isolated strain was carried out according to modified Kirby Bauer disc diffusion method. In our present study one strain out of 55 MRCONS was resistant to Vancomycin by broth dilution method.

**Conclusion:** From present study it may be concluded that rapid and reliable identification up to species level is necessary in order to predict early diagnosis of infection & Antibiotic susceptibility pattern of each clinical isolate is equally important to initiate treatment.

**Keywords:** Vancomycin, methicillin, neonatal intensive care unit

Introduction:

Recently due to the improvements in the treatments of serious diseases, there are more and more patients whose resistance to infection is severely reduced. In recent years CoNS has emerged as a pathogen in growing number of serious nosocomial infections. They are an important cause of infection in hospitalized patients who are immunocompromised and or are suffering from chronic diseases. This is particularly true in the blood stream infections of Neonatal Intensive Care Unit [NICU] patients. Treatment of these infections caused by CONS, is increasingly problematic due to frequent occurrence of multiple antibiotic resistance. Hence, rapid and reliable identification up to species level is necessary in order to predict early diagnosis of infection. Antibiotic susceptibility pattern of each clinical isolate is equally important to initiate treatment. It has also been found that Methicillin resistance is even more prevalent among CONS, which leads to increased use of Vancomycin as empirical therapy. With the background in mind we planned the present work to...
study of prevalence of methicillin and vancomycin resistance in multidrug resistant Coagulase Negative Staphylococci.

Materials & Methods:
The present study was conducted in neonatal intensive care unit (NICU) of a tertiary care hospital over a period of one year. A Total of 972 neonates both preterm and fullterm, who were clinically suspected of having sepsis were included in the study. Blood, Pus, CSF and Urine specimen were collected. These samples were processed by standard bacteriological techniques. 269 samples from NICU environment were also collected during same time and period. Direct microscopy & blood culture was done .

Antimicrobial susceptibility Test: Antimicrobial susceptibility testing of the isolated strain was carried out according to modified Kirby Bauer disc diffusion method. The suitable strength of the antibiotic disks used. All the disks used was of Hi-Media Mumbai. The results of the test are interpreted as sensitive and resistant as per CLSI guidelines Oxacillin disc was used to find methicillin resistance (CLSI 2007). The disc diffusion method is reliable for detection of methicillin resistance if the proper antibiotic and temperature are used. In present study work isosensitest agar with first line set of discs incubated at 30°C for 24 hr. was used.

Observations & Results:

Table 1: Methicillin resistance by disc diffusion method

<table>
<thead>
<tr>
<th>Total no. of CONS</th>
<th>Methicillin resistant</th>
<th>Methicillin sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONS</td>
<td>CONS</td>
</tr>
<tr>
<td>100</td>
<td>58</td>
<td>42</td>
</tr>
</tbody>
</table>

In our present study there was seen 58% of CoNS isolated showed Methicillin resistance while 42% were sensitive to Methicillin by disc diffusion method.( table 1)
Table 2: Minimum inhibitory concentration (MIC) of Vancomycin for MRCONS isolates:

<table>
<thead>
<tr>
<th>MIC Value of Vancomycin µg/ml</th>
<th>Number MRCONS strains (n = 55)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive</td>
<td>0.25</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Intermediate</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Resistant</td>
<td>&gt;32</td>
<td>1</td>
</tr>
</tbody>
</table>

Discussion:
Coagulase negative staphylococci (CoNS) are the major cause of nosocomial infections\(^7\). Since 1950, infections with these organisms are being reported with increasing frequency. Their role in human pathology has been largely accepted. In our study, we have collected samples like blood, CSF, pus and urine from septicaemic neonates. We have tested methicillin resistance by using oxacillin disc. We got 58% MRCoNS strains by disc diffusion method. The continuously high prevalence of methicillin resistant staphylococci (MRS) throughout the world is constant threat to public health, owing to the multiresistant characteristics of these bacteria. Available data accord with the supposition that the genetic basis, expression and regulation of methicillin resistance in coagulase negative staphylococci are similar to those in methicillin resistant S. aureus\(^7\).

In staphylococci, methicillin resistance may appear either as a homoresistant or a heteroresistant trait. Haretman and Tomasz defined homoresistance when >1% of the cell population grew on tryptic soya agar supplemented with 50ug/ml of methicillin and incubated at 37°C for 72-96 hr. Hetroresistance was defined when <1% of cells grew on agar plates. A uniformly high level of resistance appear among the cell in homoresistant strains, while two subpopulations of cells appear in hetroreistant strains one population of
the few highly resistant cells, and a majority of the cells which are fairly susceptible to methicillin.

Vancomycin resistant S. haemolyticus causing bloodstream infections have been reported by et al from Iowa. O’Hare et al have found in teicoplanin-resistant, vancomycin sensitive S. epidermidis, a 39 KDa protein fractionation of cell components showed that this protein was present in the membrane fraction. Investigation with S. haemolyticus revealed the presence of a 35KDa protein in membranes of resistant strain. The amount was increased substantially by growth in sub-inhibitory levels of teicoplanin. But the function of an additional protein in S. epidermidis and S. haemolyticus and their possible role in glycopeptide resistance remains to be elucidated.

Different workers have shown different antibiogram for CoNS isolated from NICU. Amita Jain et al (2002) – Penicillin 89.6%, Erythromycin 43%, Cotrimazol 68.8%, Ciprofloxacin 43%, Gentamicin 58.4%, Tetracycline 53.3%, Amikacine 3.4%. They showed 100% sensitivity to teicoplanin and vancomycin. This could be because of prophylactic antibiotics used in neonates on admission. Kumhar G.D. et al (2002) showed sensitivity to vancomycin 80%, this high resistant to vancomycin may be due to that, their study included only 26 CoNS isolated, further study of more number of isolates should be done for interpretation.

Methicillin Resistant in CONS:
In our study of 100 CoNS isolated from NICU, 58 were methicillin resistant by disc diffusion method. This is in correlation with the finding of Amita Jain et al who showed isolation of MRCONS 66% from 660 neonatal septicemia cases. The study of Christian C. Patric et al (1999) have got 70% MRCONS isolation from 73 CoNS they studied. Kumhar G.d. (2002) et al showed isolation of 80% MRCoNS out of 78 isolates. Methicillin resistant Staphylococci spp. present in hospital personal may act as carrier and can serve as a focus for nosocomial spread of multidrug resistant staphylococci in tertiary level hospital. This may result in problems in for hospital infection control programmes. The percentage of methicillin resistance increases with hospital stay and with exposure to antibiotics, particularly the semisynthetic penicillines.

The routine method of antimicrobial susceptibility testing of oxacillin using Kirby-Bauer Disk Diffusion method has certain limitations. It must be performed under certain conditions otherwise strains can be mislabeled as MRCoNS. Misidentification of strains can have detrimental consequences on patient’s management, prevention and control. If false resistance results were found, it could lead to use of inappropriate and toxic antimicrobial agents. Thus it is important to confirm MRCONS strains, which were identified by disc-diffusion method and further confirmed by more accurate MIC method. In our study, we studied all 58 Methicillin resistant CoNS strains (identified by conventional disc-diffusion test) using tube dilution method of Minimum inhibitory concentration (MIC) of CoNS strains with MIC of oxacillin $\geq 4 \mu g/ml$ are considered methicillin resistant while susceptible strains have MIC of $<4 \mu g/ml$.

In the present study 3 isolates which were resistant by disc diffusion method were found to be sensitive by the MIC method, majority of isolates 18 (31.03%) were found to have MIC value of oxacillin 32 $\mu g/ml$, 12 (20.6%) 8 $\mu g/ml$ and 9 (15.1%), 64 $\mu g/ml$. Other worker, Aftab R et al also showed Methicillin resistant by MIC was 64% with the MIC value of $>32 \mu g/ml$ in their study of 36 CoNS from neonates. Until recently, Vancomycin was the only uniformly effective treatment available for MRCONS infections, but the inadvertent and inappropriate use of vancomycin has
given rise to the emergence of vancomycin resistant strain. In the present study broth dilution assay (tube dilution method) was used to detect MIC of vancomycin. We found that out of 55 MRCONS, one strain was resistant to vancomycin at >32 µg/ml dilution by both disc-diffusion and MIC method.

Kumhar G.D. et al (2002) in their study of 1828 neonates, in gram positive organism they got 20% resistant to vancomycine, this may be explained by their small amount of CoNS they have followed. However, other worker, Christian Patrick C et al (1989), Roy have showed 100% sensitivity to vancomycin to CoNS isolated from the neonates. This can be explained by the fact that pattern of antibiotic resistance changes according to epidemiological area. In future this knowledge can be improved with help of innovative technologies and their applications in medical education. Techniques like data mining can be more helpful in assessment and diagnosis.

**Conclusion:** From present study it may be concluded that rapid and reliable identification up to species level is necessary in order to predict early diagnosis of infection & Antibiotic susceptibility pattern of each clinical isolate is equally important to initiate treatment.

**Abbreviations:**

CoNS : Coagulase Negative Staphylococci
NICU : Neonatal Intensive care unit

**References:**


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