Species-wise distribution of coagulase negative staphylococci from various clinical specimens by standard bacteriological techniques.

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Abstract:

Introduction: In recent years coagulase negative staphylococci 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. With this background in mind, the present study was planned to study the Species-wise distribution of coagulase Negative Staphylococci from various clinical specimens by standard bacteriological techniques.

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 full term, who were clinically suspected of having sepsis were included in the study. The written informed consent was obtained. Blood, Pus, CSF and Urine specimen were collected.

Observations & Results: Culture positive seen in 348 neonates (35.80%) while Culture negative seen in 624 neonates (74.20%). A total of 494 isolates were obtained from 348 neonates. CONS isolated were 100 out of 494 organisms (20.24%) while other organisms isolated were 394 out of 494 organisms (79.66%).

Conclusion: From the present study we may conclude that the presence of high rate of blood culture positivity in our study and other workers studies shows the importance of blood culture in diagnosis of neonatal septicemia.

Keywords: Coagulase Negative Staphylococci, blood culture, Neonatal Intensive care unit

Introduction:
Coagulase negative Staphylococci [CoNS] historically have been regarded as saprophytes with little pathogenic potential. However, it is now well known that under appropriate conditions, they can produce serious human diseases. CoNS are pathogenic when alterations in the integument allow these normal skin inhabitants to gain entry into the body. 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. In more than 50% cases CoNS has been isolated. Skin colonization by CoNS can be demonstrated in over 90% of NICU admissions; these organisms may then gain entry to the blood during an invasive procedure and result in sepsis. With this background in mind, the present study was planned to study the Species-wise distribution of coagulase Negative Staphylococci.
from various clinical specimens by standard bacteriological techniques.

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. The written informed consent was obtained. Blood, Pus, CSF and Urine specimen were collected. All the specimens were brought to the laboratory within two hours of collection and further processing was done.

A) Direct Microscopy: Gram staining was done. Smears were then examined for various morphological types of organisms and presence or absence of pus cells.

B) Culture: Specimens were inoculated onto nutrient agar, blood agar and Mac-Conkey agar plates. The plates were incubated aerobically, at 37°C for 24 hours. After 24 hours plates were observed for the growth, colony morphology. Colonies were picked up from culture and gram staining was done. Gram positive cocci, arranged in clusters, pairs were further considered for the study. Staphylococci were differentiated from Micrococci with the help of three tests, Furazolidone susceptibility (100 µg), Bacitracin susceptibility (0.04 µ) & Modified oxidase test – Modified oxidase reagent is prepared just before use by adding dimethyl sulfoxide(DMSO) in Teramethyl-p-phenylenediamine dihydrochloride(oxidase reagent).

The gram positive cocci, furazolidone (100 µg) sensitive, bacitracin resistant and modified oxidase test negative, novobicin sensitive and Anaerobic glucose fermentation positive were considered as staphylococci and further studied.

Then on these colonies, following biochemical reaction were done:
1) Tube coagulase test
2) Urease test
3) Carbohydrate fermentation test: Glucose, Sucrose, Mannitol, Maltose, Xylose

Observations & Results:

Table 1: Growth obtained from specimens collected from 972 septicemia neonates:

<table>
<thead>
<tr>
<th></th>
<th>Culture +vet</th>
<th>Culture –vet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total neonates</td>
<td>972</td>
<td>348</td>
</tr>
<tr>
<td>Total specimens collected</td>
<td>1142</td>
<td>494</td>
</tr>
<tr>
<td>CONS</td>
<td>100</td>
<td>Other</td>
</tr>
<tr>
<td></td>
<td></td>
<td>organisms</td>
</tr>
<tr>
<td></td>
<td></td>
<td>394</td>
</tr>
</tbody>
</table>

In our present study total 1142 specimens were collected from 972 neonates. Culture positive seen in 348 neonates (35.80%) while Culture negative seen in 624 neonates (74.20%). A total of 494 isolates were obtained from 348 neonates. CONS isolated were 100 out of 494 organisms (20.24%) while Other organisms isolated were 394 out of 494 organisms (79.66%).
Table 2: Different organism and their frequency of isolation from different clinical specimens:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Blood</th>
<th>Pus</th>
<th>CSF</th>
<th>Urine</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>52</td>
<td>29</td>
<td>5</td>
<td>29</td>
<td>115</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>59</td>
<td>43</td>
<td>2</td>
<td>10</td>
<td>114</td>
</tr>
<tr>
<td>CONS</td>
<td>58</td>
<td>28</td>
<td>12</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>S. aureus</td>
<td>36</td>
<td>14</td>
<td>6</td>
<td>2</td>
<td>58</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>29</td>
<td>18</td>
<td>8</td>
<td>1</td>
<td>56</td>
</tr>
<tr>
<td>Candida</td>
<td>14</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td>Citro</td>
<td>12</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>260</td>
<td>147</td>
<td>40</td>
<td>47</td>
<td>494</td>
</tr>
</tbody>
</table>

From table number 2, it is seen that out of 494 isolates E. coli was the major isolate 115 (23.27%), followed by Klebsiella spp. 114 (23.07%), Coagulase negative Staphylococci 100 (20.24%). From CSF 40 specimens, CoNS was the major isolate 12 (30%). In 260 suspected cases of neonatal septicemia 58 were caused by CoNS. In 147 suspected cases of skin infection comprising of pyoderma, umbilical stump discharge, inflammation at site of catheter tip, conjunctivitis 28 were caused by CoNS. In 40 cases of suspected meningitis 12 were caused by CoNS. In 47 suspected cases of Urinary tract infections 2 were caused by CoNS.

Discussion: Coagulase negative staphylococci (CoNS) are the major cause of nosocomial infections. Since 1950, infections with these organisms are being reported with increasing frequency. Their role in human pathology has been largely accepted. Coagulase negative staphylococci are opportunistic pathogens that cause infections in debilitated or compromised patients such as premature neonates, cancer patients, burn patients, end stage renal disease, often by colonizing biochemical devices such as prostheses, implants and intravascular lines. Nosocomial infections are widespread. They are important contributors to morbidity and mortality. Neonates are particularly vulnerable to infections because of their weak immune status.

In the present study 100 CoNS strains were isolated from various clinical specimens received from neonates in NICU of a tertiary care hospital. Also 39 CoNS strains were isolated from NICU environment during same period. In our study positive growth was seen in 43.25% of specimen collected. This study correlates with the study of Roy et al (2002) who got growth in 346 (47.5%) out of 728 specimens. In our study, we have collected samples like blood, CSF, pus and urine from septicemic neonates. As is clear from table 2, blood showed maximum number of growth positivity, i.e. 260 (54.3%) out of 494 positive samples. Our finding is comparable to that of Villari P. et al (2000), who got blood culture positive in 47.8% among 184 infected neonates blood sample. Other workers Roy I. et al (2002) 47.5%, and Kumhar G.D. et al showed that 42% of blood cultures were positive out of 1828 samples. There are some workers who showed less percentage of blood culture positivity. Barbar Stoll M.D. et al studied 6215 infants and obtained...
blood culture positivity in 1313 (21%) infants. This could be the possible that their high level of NICU set ups prevents nosocomial inf In our study we got 147 (29.7%) pus samples culture positive. These results are comparable to the finding of Villari P. et al (256) who got 25.5% pus culture positive out of 1010 samples.

In this present study 40 (8.1%) CSF samples showed positive growth out of 497 samples. Our results are comparable with that of Villari P. et al who had shown growth in 6.5% CSF samples. 

47 urine samples out of 494 samples were culture positive (9.5%), other workers also have a comparable growth from urine. Villari P. and Sarnataro showed 8.7% growth from total of 1010 samples. From 494 growth positive samples we got E. coli 115 (23.17%) from all the specimens collected. Klebsiella spp. 114 (23.07%) followed by CoNS (20.24%) and S. aureus 11.74%. Whereas others like, Kumhar G.D. et al showed predominant isolation of Klebsiella (33.8%) followed by E. coli (4.6%) and CoNS 7.9% in their study of 1828 neonates. Patric C. et al studied 136 neonates and isolated Klebsiella 13%, E. coli 7%.

In the study done by Wankhede et.al., Esch.coli (5%) and K. Pneumoniae (8.33%) MBL producers were detected. In India presence of MBL producers in other gram negative bacteria are not reported much. A study done by Balan et al (2012) found that total of 500 serum samples from people of different age group attending the outpatient department of our hospital were studied to see the presence of heterophile antibodies against RBC of rabbit, guinea pig, sheep and fowl. Out of these 500 sample, 252(50.4%) were from females and 248(49.6%) were from males. In the present study 100 CoNS were isolated from 494 organisms, which amount to 20.24%. Comparable studies show that, 16.5% of CoNS were isolated from neonatal septicemic cases out of 346 organisms in 728 neonates by Roy I. et al; similarly 16.4% of CoNS were isolated from neonatal septicemia out of 660 organisms by Amita Jain et al. (2004) Ni-hung Lee et al (2004) have isolated 29% CoNS from 87 organisms isolated. This high rate of isolation could be explained on the basis that majority of these organisms were from neonates with very low birth weight and their sample collection was after the 3 days of admission.

Conclusion: From the present study we may conclude that the presence of high rate of blood culture positivity in our study and other workers studies shows the importance of blood culture in diagnosis of neonatal septicemia.

Abbreviations:
CoNS : Coagulase Negative Staphylococci
NICU : Neonatal Intensive care unit

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15. Dr. Wankhede S.V., Dr. Iyer V.S., Dr. Bharadwaj R.S. The study of MBI producers in gram negative isolets from ICUs and wards. Indian Journal of Basic & Applied Medical Research; December 2011: Issue-1, Vol.-1, P. 38-46