Original Research Article

Evaluation of Aerobic Bacterial Flora from Environment, Infected Neonates and Health Care Personnel in a Neonatal Intensive Care Unit

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ABSTRACT

Hospital acquired infections among neonatal intensive care unit have been linked with many risk factors like microbial flora of NICU environment, bacterial colonisation of its inhabitants and patients. The present study was carried out with to study the aerobic bacterial flora of NICU Environment, NICU patients and HCWs.

The most commonly isolated organism was Coagulase Negative Staphylococcus (CoNS) followed this by Multidrug resistant (MDR) Klebsiella pneumoniae. Umbilical swabs, Environmental air, ventilators, air-conditioners, X-RAY machine and ECG machine are the sites of isolating Methicillin-resistant Staphylococcus aureus (MRSA), MDR Klebsiella pneumoniae and AmpC Pseudomonas aeruginosa.

The results of the study emphasize the need of Continuous microbial surveillance for colonisation and risk factors identification. Infection control practices and antibiotic policy should be strictly followed in NICU.

Key-words: hospital acquired infection (HAI), Neonatal intensive care unit (NICU), Health care worker (HCW), Multidrug resistant (MDR), Colonisation.

INTRODUCTION

Healthcare-associated bloodstream infection is a serious problem in neonatal intensive care unit (NICU). Despite best efforts promoting infection control protocols, hospital-acquired infections (HAI) remain a common complication of hospital care, occurring at an estimated rate of 2 million infections annually in the United States. The rate is more in developing countries. [1]

Microbes have an inherent ability to colonize any surface. An important risk factor for nosocomial infection is prior colonization. Studies have shown that microbes can persist for weeks on stainless steel surfaces and polymeric materials used to fabricate touch surfaces in hospitals. Methicillin-resistant Staphylococcus aureus (MRSA) may exist on surfaces for as long as 360 days and spore-forming bacteria, including Clostridium difficile, can survive for months. The longer a nosocomial pathogen persists on a surface, the longer it may be a source for transmission to a
susceptible patient or health care worker (HCW).\textsuperscript{[2,3]}

Immature host defense mechanisms and invasive life support systems make the neonate particularly susceptible to overwhelming infections. The large use of antimicrobials drugs and cross transmission via the hands of care givers, contaminated equipments, or environment plays a prominent role in causation and dissemination of infections.\textsuperscript{[3,4]}

The present study aims to find out the aerobic bacterial flora of NICU Environment, patients in NICU and HCWs.

**MATERIAL AND METHODS**

The study was conducted by the department of Microbiology, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha & NICU, Acharya Vinoba Bhave Rural Hospital, Sawangi (Meghe), Wardha. The study period was 6 months from January to June 2014. The area of NICU is 600 sq. ft with 11 radiant warmers, 4 ventilators and 3 Phototherapy units. There are 4 air conditioners, 13 suction machines (central) and other necessary equipments.

Study was conducted after obtaining approval from Institutional ethical committee.

The samples were collected from three sources

1. Environment
2. Health care providers working in NICU
3. Infected Neonates

**Collection and Processing Of Samples:**

**Environmental Air Sampling:** Sedimentation method using open petri dishes containing different culture media was used for air sampling weekly. Two plates (blood agar and Mac Conkey agar) were exposed (kept open) for 30 minutes in NICU at different places. After this plates were covered and transferred to microbiology laboratory. Plates are incubated at 37\(^\circ\)c for 24 hours. The number of colonies counted. The identification of the isolates was done according to standard microbiology procedures.

The following samples were collected monthly from NICU. Sterile swabs moistened with sterile saline were used for collection.

- **Environmental Swab:** from Oxygen hood, Neonatal resuscitator, Defibrillator, Multiparamonitor Pulse oximeter, ECG machine, X-ray machine, Baby Trolley, Radiant warmer with trolley, Air conditioners, phototherapy units, IV stands, suction machines etc

- **Health Care Provider’s Swabs:** After taking written consent nasal swabs, hand swabs and pen/mobile swabs of health care workers who had direct contact with neonates hospitalised in the NICU were collected.

- **Neonatal Swabs:** Nasal swab, skin swabs and umbilical swabs of admitted infected neonates were taken after obtaining written consent from their parents.

All swabs (except nasal swabs of NICU inhabitants) were inoculated on Blood agar and Mac Conkey agar. For nasal swabs of NICU inhabitants mannitol salt agar was used to screen for Staphylococcus aureus. The identification of the isolates was done according to standard microbiology procedures.

All the isolates were subjected to in vitro antibiotic susceptibility testing by disc diffusion test on Muller-Hinton agar as per CLSI guidelines 2012. Interpretation of antibiotic susceptibility was done using Himedia interpretation chart and following CLSI guidelines 2012.

**RESULTS**

During study period we collected 227 samples from different sources. We
isolated 83 bacteria. With more than one bacterium from some swabs. All the umbilical swabs showed multibacterial growth. Table 1 depicts the data of various bacteria isolated from different sites. The most commonly isolated organism was Coagulase Negative Staphylococcus (CoNS) 27/83 (32.53%) followed this by Multidrug resistant (MDR) Klebsiella pneumonia 26/83 (31.33%).

Growth of MDR Klebsiella pneumoniae and Methicillin resistant Staphylococcus aureus were cause of concern. With growth of MDR Klebsiella pneumoniae from 11/22 (50%) umbilical swabs. Methicillin resistant Staphylococcus aureus were isolated most commonly from 4/18 (22.22%) environmental air sample. Among the environmental swabs ventilators, air-conditioners, X-RAY machine and ECG machine were the sites of isolating MDR Klebsiella pneumoniae and AmPc Pseudomonas aeruginosa.

Antibiotic susceptibility testing of CoNS isolates showed different sensitivity patterns. All isolates were sensitive to vancomycin. Higher sensitivity was observed to amikacin and least to oxacillin, penicillin, erythromycin and gentamicin.

Among all MDR Klebsiella pneumoniae isolates 20 were carbapenamase producing and 6 AmPc producing.

Table 1: Organisms isolated from different sources

<table>
<thead>
<tr>
<th>Organisms isolated</th>
<th>Environmental Air samples(N=18)</th>
<th>Environmental Swabs(N=80)</th>
<th>NICU HCWs(N=21)</th>
<th>Admitted neonates(N=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nasal swabs</td>
<td>Hand swabs</td>
<td>Mobile/pen swabs</td>
<td>Nasal swabs</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>AmPc Pseudomonas aeruginosa</td>
<td>0</td>
<td>1</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas Species</td>
<td>3</td>
<td>2</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0</td>
<td>1</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Coagulase Negative Staphylococcus</td>
<td>3</td>
<td>7</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Methicillin Resistant Coagulase Positive Staphylococcus</td>
<td>4</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Methicillin sensitive Coagulase Positive Staphylococcus</td>
<td>0</td>
<td>1</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Acinetobactersp</td>
<td>1</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Total isolates</td>
<td>14</td>
<td>15</td>
<td>01</td>
<td>08</td>
</tr>
</tbody>
</table>

DISCUSSION
Hospital acquired infections among neonatal intensive care unit have been linked with many risk factors like microbial flora of NICU environment, bacterial colonisation of its inhabitants and patients. This emphasizes the need to determine the spectrum of bacterial colonisation in NICU.

In the present study the most commonly isolated organism was Coagulase Negative Staphylococcus. These findings correlates well with findings of study in Cairo where CoNS were the most commonly isolated organisms from superficial cultures, hand swabs of HCWs and blood culture in NICU. [4]

In this study hand s of Health care workers were contaminated in 8/21 (38.1%) with Escherichia coli, and CoNS as common isolates. CoNS skin carriage among Neonatal Intensive Care Unit Personnel was also reported in study at Netherlands and Iran.[5,6]

In study at New York Gram-negative bacilli were isolated at least once from the hands of 45 (38%) of 119 nurses despite the fact that cultures of nurses hands were done after hand hygiene was performed. They
found a greater risk of horizontal transmission via hands with certain gram-negative bacteria (*K. pneumonia* and *S. marcescens*). They suggested that, although proper hand hygiene practices are clearly important, hand hygiene alone may not be sufficient to prevent horizontal transmission of gram-negative pathogens seen in the NICU. [7]

When we studied the spectrum of bacterial colonisation in patients it was found that bacterial colonisation of one or more sites was detected in 12/22 (54.55%) with *Klebsiella pneumoniae* a most common isolate. This finding correlates well with the finding of study of colonisation in patients of ICU at Lucknow where bacterial colonisation in nasal, oral and rectal swabs was detected in 51/96 (53.13%) patients. [3] Similarly In the study at NICU in Philippines more than half (55.5%) neonates colonised with drug resistant gram negative bacilli during NICU hospitalization. Common gram negative bacilli were *Klebsiella* species, *Pseudomonas aeruginosa* and Enterobacter species. [8]

Isolation of MDR *Klebsiella pneumoniae* in 11/22 (50%) umbilical swabs and MRSA in 4/18 (22.22%) environmental air samples was cause of concern. These are important hospital acquired pathogens with the potential of causing severe morbidity and mortality in neonates. The therapeutic options to the infections caused by these organisms are limited because of their resistance to multiple antibiotics. Colonisation by these organisms in NICU was observed by many researchers. In study at NICU in China ESBLK Klebsiella pneumonia was yielded in 14.9% environmental samples, 4.5% in colonized neonates, 2.6% infected neonates and also hands of HCWs. [9] Seven year weekly surveillance programme for MRSA colonisation of nasal/rectal swabs in infants and cases was carried out at tertiary care NICU in Boston. MRSA isolates with 14 different antibiograms were found during the study period. There was a shift from isolates predominantly likely to be hospital-associated in 2000–2004 to those likely to be community-associated in 2006–2007. It was observed that multiple MRSA strains were introduced into the NICU over time. [10]

**CONCLUSIONS**

Colonisation with resistant pathogenic flora was seen in NICU environment, patients and health care workers. Hand hygiene, environmental hygiene, care of umbilical cord is mandatory for infection control in neonatal intensive care units. The results of the study emphasis the need of Continuous microbial surveillance for colonisation and risk factor identification. Infection control practices and antibiotic policy should be strictly followed in NICU.

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