Antimicrobial Resistance Patterns of Colonizing Microflora on the Personnel Hands and Noses Working in the Neonatal Intensive Care Unit (NICU)

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Abstract: Background: The normal flora of health care workers may serve as reservoirs for causing health care-associated infections in neonatal intensive care units (NICUs). The aim of the present study was to assess the aerobic microflora on the hands and noses of nurses and non-patient care workers (NPCWs) and their antibiotic resistance patterns. Methods: During a three-month period, samples were obtained from 48 nurses and 16 NPCWs. After identification of bacteria, antimicrobial susceptibility against 8 antibiotics was performed according to CLSI recommendations. In addition, to detect inducible clindamycin resistance (iMLS\(_{\text{B}}\)), double disk diffusion (D-test) was carried out. Results: Among microbial isolates recovered from our participants, the commonest isolates among nurses and NPCWs were coagulase-negative staphylococci (CoNS) with prevalence of 74% and 90%, respectively. Methicillin resistant staphylococci in nurses in comparison to NPCWs were significantly higher (P < 0.0001). Moreover, more iMLS\(_{\text{B}}\) phenotypes were found among CoNS isolated from NPCWs than nurses. Conclusion: In regard to higher rate of resistant bacteria shown in nurses compared to NPCWs and direct contact nurses with neonates and exposure to the hospital environment, it appears that in addition to hand hygiene, other interventions such as using sterile gowns and disposable gloves by personnel are necessary.

Key words: Antimicrobial resistance • Microflora • Hand hygiene • Patient care workers • NICU • Coagulase-negative staphylococci (CoNS)

INTRODUCTION

Nosocomial infections (NIs) are regarded as the main global concern and their prevalence rates are variable based on some factors, however, about one–third of NIs are preventable. The most important method for NI control is hand hygiene [1-3], because hand washing helps reduce the transmission of skin microflora originating from hand and nose of patient care workers (PCWs), particularly the nurses [1,4]. These microorganisms includes resident, transient and infectious flora [3]. Moreover, the flora of the hands of PCWs vary by work setting and differ from non-patient care workers (NPCW) [3,5]. Factors such as temperature, moisture and work environment can affect the number and types of flora found on PCWs hands [5]. As the nurses are in direct contact with neonates, the neonatal intensive care units (NICUs) are at high risk of Nis [1,6]. According to literature, coagulase-negative staphylococci (CoNS) are the most common bacteria associated with NICU infections and most of them show multi-drug resistance (MDR), such as methicillin [6-8]. For the above reasons, this study aimed at determining the most common aerobic flora recovered from the hands and noses of NICU nurses and NPCWs and their antimicrobial resistance patterns in our region.

These authors contributed equally to this work.

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**MATERIALS AND METHODS**

**Sample and Setting:** At this cross-sectional survey, during a three-month period (December 2011 through March 2012) all full-time nurses working in six Shiraz hospitals, which had NICU ward were asked to participate in the study. Of them, 48 subjects (>80%) agreed to participate. A consent form was obtained from all participants before sampling. Exclusion criteria were individuals who had been treated with antibiotics within 7 days before specimen collection. Indeed, 16 NPCWs (i.e. accounting and administrative personnel) were included as the control group.

During the study all the nurses were using the same hand hygiene product. The hands sampling were taken from the subjects using a sterile swab moistened with sterile physiologic saline. For this purpose, the entire surface of the palm of the personnel’s dominant hand was examined. For nasal sampling, sterile swab premoistened with sterile physiologic saline was used to both anterior nares by rotating 4-5 times around the inside of each nostril with even pressure. All samples from both groups were obtained during the work day. This study was approved by the Medical Ethical Committee of Shiraz University of Medical Sciences (CT-90-5574).

**Culture and Bacterial Identification:** After taking the samples, the swabs were transferred in tubes containing tryptone soy broth (Merck, Germany) and in laboratory were plated onto the tryptone soy sheep blood agar (5%) and MacConkey agar (Merck, Germany). All plates were incubated aerobically 48 h at 37° C. Bacterial identification was done by conventional microbiologic procedures.

**Antibiotic Susceptibility Testing:** This was performed on staphylococcal isolates by disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) recommendations [9]. The antibiotic disks of ampicillin, clindamycin, erythromycin, gentamicin, rifampin, tetracycline, trimethoprim/sulfamethoxazole and vancomycin (Neo-Sensitabs™, Rosco, Denmark) were used. A cefoxitin disk (30 µg) was used for screening of methicillin susceptibility of all staphylococcal isolates. Also, for determining of inducible clindamycin resistance among staphylococci that were erythromycin-resistant (ERY-R) and clindamycin-susceptible (CLI-S), double disk diffusion (D-test) was performed as described by CLSI. For quality control *Staphylococcus aureus* ATCC 25923 was used. Isolates were considered as sensitive or resistant according to CLSI. We categorized the intermediate and resistant isolates as resistant.

**Data Analysis:** Statistical analysis was performed using SPSS, version 19.0. Chi–square test was conducted to analyze the rates of antibiotic resistance in both groups’ subjects. Values of p < 0.05 were regarded as statistical significance.

**RESULTS**

A total of 147 and 52 microbial isolates were recovered from the swabs of hand and nose of the nurses and NPCWs, respectively (Figure 1).

As shown, the three types of resident (CoNS and Diphtheroids), transient (*Micrococcus roseus*, *Bacillus* spp., *Entrococcus* spp., á- hemolytic streptoccci spp., *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae*) and infectious flora (*S. aureus*) were isolated from the participants. The commonest isolates in both nurses and NPCWs subjects were CoNS with prevalence of 69.4% and 90.3%, respectively. Also, the lowest rate observed among nurses was 0.7% (*Micrococcus roseus*, *E. coli* and *E. cloacae*), whereas in NPCWs it was 1.9% (*E. coli*).

Antibiotic resistance frequencies of CoNS in our survey are depicted in Table 1. In our work, all staphylococci in both groups were sensitive to vancomycin, while methicillin resistance was remarkably different (Table 2) and this difference was significant (P < 0.0001).

On the other hand, most of CoNS obtained from nurses exhibited multi-drug resistant (MDR) phenotype (P < 0.0001), which in both groups were mainly triple or quadruple resistance with frequencies of 43 (42%) and 12 (25.5%) in nurses and NPCWs, respectively. In 8 (7.8%) isolates, resistance against all used antibiotics, except vancomycin, among nurses was shown. With increased number of disks, the frequencies of MDR in NPCWs isolates were diminished.

Moreover, it was found out that 10 (13.5%) methicillin-resistant coagulase-negative staphylococci (MRCoNS) isolates obtained from nurses were ERY-R (MS₉ phenotype) and 53 (69.73%) of them exhibited resistance constitutive clindamycin resistance (resistant to both ERY and CLI = cMLS₉ phenotype). Inducible clindamycin resistance (iMLS₉ phenotype) among nurses was detected only in one (3.84%) methicillin-susceptible coagulase-negative staphylococci (MSCoNS) isolate. Another notable result was the presence of more iMLS₉ phenotypes in CoNS isolated from NPCWs than nurses. Table 3 summarizes the different types of this resistance among our isolates.
Table 1: Antibiotic resistance pattern of CoNS isolates recovered from both personnel groups

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>CoNS recovered from hands n (%)</th>
<th>CoNS recovered from nasal n (%)</th>
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<tbody>
<tr>
<td></td>
<td>Nurses (n=59)</td>
<td>NPCWs (n=32)</td>
</tr>
<tr>
<td>Tetracycline (30µg)</td>
<td>30 (50.8%)*</td>
<td>8 (25.0%)</td>
</tr>
<tr>
<td>Erythromycin (15µg)</td>
<td>54 (91.5%)*</td>
<td>18 (56.2%)</td>
</tr>
<tr>
<td>Clindamycin (2µg)</td>
<td>42 (71.1%)*</td>
<td>8 (25.0%)</td>
</tr>
<tr>
<td>Gentamicin (10µg)</td>
<td>21 (35.5%)*</td>
<td>0</td>
</tr>
<tr>
<td>Trimethoprim/ sulfamethoxazole (25µg)</td>
<td>28 (47.4%)*</td>
<td>1 (3.1%)</td>
</tr>
<tr>
<td>Rifampin (5µg)</td>
<td>3 (5.0%)</td>
<td>0</td>
</tr>
<tr>
<td>Ampicillin (10µg)</td>
<td>51 (86.4%)*</td>
<td>16 (50.0%)</td>
</tr>
<tr>
<td>Vancomycin (30µg)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefoxitin (30µg)</td>
<td>46 (77.9%)*</td>
<td>10 (31.2%)</td>
</tr>
</tbody>
</table>

CoNS: coagulase-negative Staphylococci, NPCWs: None-patient care workers

* p-value was significant (p<0.05)

Table 2: Distribution of MRCoNS found on the hand and nose of both personnel groups

<table>
<thead>
<tr>
<th></th>
<th>Nurses (n=102)</th>
<th>NPCWs (n=47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand Resistant No. (%)</td>
<td>47 (79.6%)</td>
<td>8 (25%)</td>
</tr>
<tr>
<td>Nasal Resistant No. (%)</td>
<td>29 (67.4%)</td>
<td>3 (20%)</td>
</tr>
</tbody>
</table>

PCWs: None-patient care workers.

Table 3: MLS\textsubscript{B} resistance pattern of MRCoNS and MSCoNS obtained from both personnel groups

<table>
<thead>
<tr>
<th>MS\textsubscript{B} phenotype</th>
<th>cMLS\textsubscript{B} phenotype</th>
<th>iMLS\textsubscript{B} phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>MRCoNS</td>
<td>MSCoNS</td>
</tr>
<tr>
<td>Nurse % (No.)</td>
<td>13.5(10)</td>
<td>15.38(4)</td>
</tr>
<tr>
<td>NPCW % (No.)</td>
<td>45.45(5)</td>
<td>13.88 (5)</td>
</tr>
</tbody>
</table>

MS\textsubscript{B} (ERY-R): Macrolide, Streptogramin. (Erythromycin resistant)
cMLS\textsubscript{B}: Constitutive Macrolide, Lincosamide, Streptogramin
iMLS\textsubscript{B}: Inducible Macrolide, Lincosamide, Streptogramin
MRCoNS: Methicillin-resistant coagulase-negative staphylococci
MSCoNS: Methicillin-susceptible coagulase-negative staphylococci

Fig. 1: Prevalence of different types' isolates recovered from both personnel groups
In regards of *S. aureus* strains, it was shown that all the isolates from the nurses and NPCWs were sensitive to methicillin (MSSA) and low antibiotic resistance was seen among them (data not shown). The highest resistance observed in MSSA isolates from nurses was against two agents and MS$_B$ phenotype was found in only one isolate. Indeed, no cMLS$_A$ and iMLS$_B$ phenotypes were detected among these isolates.

**DISCUSSION**

To our knowledge, this current study was probably the first survey conducted on the types, quantities and resistance pattern of microflora found on the hand and nose of the nurses and NPCWs to date in Iran. As observed, the most common bacteria isolated from both groups were gram–positive bacteria, including CoNS (69.4%) in nurses and CoNS (90.3%) and *S. aureus* (3.8%) among NPCWs. Of course, the frequency of *S. aureus* in nurses was also considerable (6.5%).

According to the literature, CoNS are the most frequent cause of different infections among NICU patients, so that they account for 50–70% of sepsis [7, 10, 11]. Several studies conducted in different regions, have shown the difference in types and antimicrobial resistance of hand or nose flora among PCWs or/and NPCWs [2, 3, 6, 12]. In contrast to some studies we found more CoNS on NPCWs as compared to nurses (p < 0.05)[2, 13, 14]. This finding is supported by Slight et al.’s study in which such a difference is attributed to long term use of disinfectants in handwashing by PCWs [12].

Also, as to *S. aureus*, our results showed that its frequency is higher on hands of medical staff than NPCWs, which is not consistent with the findings reported in previous surveys [2, 15].

In our study, the rate of recovered Gram-negative bacteria from the nurses’ hands (10.4%), was similar to that found in the study of Aiello et al. [2]. In the study of Larson et al. it is mentioned that these bacteria tend to exist in low numbers on hands of PCWs [16].

Because all the NICU staff in our study were women, NPCWs subjects were also matched with them. However, it appears that factors such as age and gender have no or limited effect on the skin microflora characteristics [17].

The best procedure for controlling the dissemination of resistant bacteria in the ICU wards is unknown. Although the studies had shown that detection and isolation of patients together with hand hygiene are effective, PCWs frequently become exposed and contaminated with resistant bacteria [6]. Nowadays, antibiotic resistance is an emerging concern in health care setting [18]. In the current study, the proportion of antibiotic-resistant CoNS, particularly in regard to methicillin (74.5%) on the nurses' hands was higher than NPCWs (23.4%). In accordance with our study, similar findings were observed by some authors [2, 12, 17, 19]. As expects, such difference about antibiotic resistance on the skin microflora of PCWs and NPCWs is likely due to the intensive care environment, where there is an extensive usage of antibiotics. This can be lead to selection and residence of resistant strains in hospital units [17]. Fortunately, all the *S. aureus* isolates from both groups were susceptible to methicillin. Indeed, none of staphylococci obtained from all subjects showed vancomycine resistance. It has been shown that methicillin-resistant and MDR-CoNS are responsible for the majority of NIs among the neonates [7, 20] and profile nosocomial organisms is shown that most of them are multi-drug resistant [21, 22]. As we showed, the rate of multi-drug resistance among CoNS isolated from nurses was significantly higher (P < 0.0001) than the control group.

In our study in contrast with a study done in the US, the marked difference in resistant CoNS recovered from the nurses’ hands versus noses isolates was not found [12].

The macrolide, lincosamide, streptogramin B (MLS$_B$) group of antibiotics are commonly used for patients with different staphylococcal infections [23]. However, the extensive usage of these agents has led to an increase in resistant staphylococci to them [24]. Diagnosis of this resistance using D-test is important, because treatment of patients harboring *S. aureus* (with iMLS$_B$ phenotype) with clindamycin can lead to development of cMLS$_B$ and ultimately therapeutic failure [25]. It has been mentioned that incidence of cMLS$_B$ and iMLS$_B$ resistance could be influenced by geographic area and even from one hospital to another hospital [23].

The iMLS$_B$ phenotype in our CoNS was 3.84% and 8.32% among nurses and NPCWs, respectively. Interestingly, none of MSSA isolates (9 from the nurses and 2 from NPCWs) demonstrated iMLS$_B$ phenotype. In the study of Eksi et al. iMLS$_B$ rate was found to be 18.6% and 11% among MRCoNS and MSCoNS strains obtained from hands of the healthcare workers, these results are higher than that of our study [3]. In a survey conducted by Rahbar et al. and colleagues of 114 CoNS recovered from clinical specimens, the frequency of iMLS$_B$ phenotype was reported 9.64% [26]. In two other studies
in Iran, prevalence of this phenotype in *S. aureus* clinical isolates was found to be 5.3% and 20.5%, respectively [25, 27].

This study has some limitations that need to be considered. Most importantly, because of using swab, we could not probably evaluate all the microflora isolates as good as modified glove-juice technique [2, 28]. Additionally, it seems that the sample size was small. However, we used all the nursing staff employed in our hospitals NICU. Furthermore, it was better that hand samples were evaluated before and after washing with their corresponding hand hygiene products. However, it appears that the washing was not effective for removing these floras [29], because in spite of daily washing of with their hand hygiene regimens, we could isolate the transient flora on their hands. This finding is supported by Slight et al.’s findings [12].

**CONCLUSION**

It seems that the most effective procedures for limiting the spread of transient bacteria, particularly MDR phenotypes from PCWs to neonates is using sterile gowns by personnel’s, disposable gloves after visiting each neonate and hand hygiene with regard to each hand hygiene product used in related units. Further research are needed to explore characteristics of persistence PCWs hand flora colonization in a period of time such as long term period absence from the hospital wards.

**ACKNOWLEDGMENT**

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