African Journal of Microbiology Research Vol. 5(16) pp. 2234-2240, 18 August, 2011
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 ©2011 Academic Journals

Full Length Research Paper

Aspects of bacterial colonization in newborn babies

Fariba Heshmati*, Seyeed Amir Yazdanparast , Seyeed Akbar Moosavi , Hussein Dargahi, and Farnaz Tabibzadeh

Department of Microbiology, Tehran University of Medical Sciences (Hemmat Campus), Tehran, Iran.

Accepted 23 June, 2011

The study of colonization of microbial flora in neonate is very important and finding the causative agent may lead to explore these infections in neonate. In this research the colonization in neonate was measured at the beginning and a few hours after birth. Sampling was performed in operating room, rooming in and nursery section. Some of the neonates deliver by rapture of membrane in normal way and the other deliver by caesarean section. All the samples which are collected were from these sites: 1) Forehead 2) Oral cavity 3) External ear 4) Auxiliary region 5) Umbilical region 6) Perennial region. Also we had some sampling from health care personnel who are worked in operating room or nursery section. At the time of sampling we used sterile gloves, mask and always sterile sets. Sampling was done by use of sterile swabs which were weltering by sterile phosphate buffer or sterile normal saline .Then this sterile swab scrubbed on the special surface of the body of neonate and directly transferred to BA plate, Mac agar plate, EMB agar plate and Sc agar plate and then streaked on these plates. The regions which were sampled ,numbered by 1 to 6 then in other hours once more the sampling were took place from the same site, therefore we began from number 7 to 12 and according to this method for example sampling from neonate A was showed by A_1 to A_{12}. The prevalent microorganisms in oral cavity were as follow: 63.5% GPC, 18.5% GPB and 11% GNC. *Staphylococcus epidermidis* was the major microorganism found in neonate forehead. No bacteria growth was found in 50% of samples through caesarean section. *Staphylococcus* and *Diphtheroied* constitute the highest colonization of neonate skin's microorganisms and their growth rates were low at the onset of the birth, but increased after 12 hours. Colonization of gram-negative bacteria in infant hours after birth considered to be an important issue that was studied in this research. Hence hand washing of health-care personnel's can reduce such bacteria in infant. There was a significant decrease in (GNB) number due to hand washing, hence application of disinfectant solution in hand washing play an important role in controlling microorganisms in care unites.

Key words: Colonization, GPC (Gram- positive cocci), GPB (Gram-positive bacilli), GNC (Gram- negative cocci), GNB (Gram- negative bacilli), Microorganism.

INTRODUCTION

Microbial colonization of newborn infant begins immediately after birth. Infants' skin is colonized by flora derived from the body of the mother and other human contacts and from various inanimate objects. Initial colonization is fortunately, depending on the first suitable organism to arrive at a particular site as well as factors such as the type of delivery, the amount of vernix caseosa persent at birth, the type of nourishment received (breast milk or formula) and the degree of exposure in the hospital environment (Guenthner et al., 1987). Moreover exclusively formula-fed infants were more often colonized with *E.coli*, *C.difficle*, *Bacteroides* and *Lactobacillus*, when compared with breast-fed infants. Other factors like hospitalization and prematurity were also associated with higher prevalence of C. difficil (Guenthner et al., 1987; Penders et al., 2006). However, in most cases, after a few days the representation of microbial species with in the neonatal flora was remarkably similar to the adult pattern of colonization.

*Corresponding author. E-mail: heshmati_99_1999@yahoo.com

Interest in the bacterial colonization of the newborn was developed in mid 1940s when epidemic of Staphylococcal pyoderma began to appear in substantial number in the developed countries (Falle and Schleifer, 1981; Pezzati et al., 2002; McConnell et al., 2004). Staphylococcal disease continues to be a serious problem in some nurseries, but gram-negative bacilli are currently the major encountered pathogens in hospital acquired infection (Pass et al., 1980). Infant born through caesarean section had lower number of *Bifidobacteria* and *Bacteroides*, but more often colonized with *C.difficle*, when compared with those borne vaginally (Penders et al., 2006). Occasionally infections are transmitted by a member of the nursery staff who harbors pathogenic bacteria on the hands or by contamination of vaginal, rectal bacteria. However, the hand transmission is more common in infant to infant (Goldmann et al., 1978). The number of nosocomial gram-negative infections has increased dramatically in the past decade but pathogenic *E.coli, Klebsiella*, *Enterobacter* and *Pseudomonas* organisms have been responsible for majority of the gram-negative infections[8]. Gram-negative bacilli (GNB) cause more than 50% of hospital acquired infections. These organisms are not thought to be part of the resident flora of the skin، perhaps because of factors such as desiccation، inhibition by skin lipids or interference by normal cutaneous bacterial flora (Guenthner et al., 1987). Most studies have assumed that GNB are part of the transient flora only and easily removed by hygienic hand washing with soap (McGarrity and Coriell, 1973). GNB remaining after removal of transient hand flora with soap and water have not been studied extensively (McGarrity and Coriell, 1973). The purpose of this study was to determine the prevalence of GNB as a part of the non transient flora on the hands of hospital personnel during the period of nosocomial infections after hand washes and also analyzing the influence of working a shift in an intensive care unit on hand carriage of GNB by nurses, the contribution of a broad range of external influences and mode of delivery on neonate's skin micro biotic composition in early infancy.

MATERIALS AND METHODS

Subjects (infants)

Twenty- two newborn infants born at A*min* hospital part of Esfahan University were included in this study. This research was conducted in 2 stages. The colonization was measured in the beginning and a few hours after birth. In the pilot study, 12 infants born by vaginal or cesarean delivery were studied. In final work, 10 infants (2 infants were premature) at operating room, rooming in and nursery section were studied.

Sampling and culture of the skin micro flora

In pilot study and final work, 96 and 80 samples were collected respectively from forehead region, oral cavity, perineum, and external ear, auxiliary and umbilical regions. Samples from the skin flora were taken 3, 4, 5, 8, 14 and 20 h after vaginal or caesarean delivery by using cotton-tipped swab weltering in sodium phosphate buffer that were scrubbed on the surface of the skin and immediately streaked on to the blood agar, Mac conkey, EMB, and Sc (sabburo cholramphenicol) plates. Samples were brought to the laboratory and incubated at 37℃ within 24 h after collection (Figure 1). Media were incubated in candle jar at 37℃ for 48 h**.** The bacterial colonies were analyzed in terms of number and morphological characteristics and then preserved in BHI and 15% glycerol for determining their species. Upon discharge from the newborn nursery at the Amin Hospital for maintaining the microorganisms, brain heart infusion broth (BHI) and 15% glycerol was used.

Figure 1. Sampling on blood agar plate.

Identification of isolates

Bacteria were stained by Gram stain and also 3% H_2O_2 solution we used to detect the catalase enzyme then identified by standard bacteriological and biochemical methods such as acid production from carbohydrates, using of MSA (Manitol salt agar medium), DNAse medium, sensivity to Furazolidon disk(100 µg), Bacitracin disk(0.04U)for differentiation *Micrococcus* from *Staphylococcus*, of medium, SIM, 10% Nacl nutrient agar, MR-VP, TSI, Simmon citrate agar, urea agar, Nitrate reduction test. Any bacterial colonies which resembled *β-hemolytic streptococci* were tested for sensivity to bacitracin with disks containing 0.04U of bacitracin, if the zone of inhibition was greater than 15 mm, the *streptococci* would presumed to be group A and then precise serological grouping was carried out. For Fungi (Candida sp) we used Sc medium and germ tube and by using of Corn meal agar medium for production of clamidospore (Figure 2).

RESULTS

In the first phase 12 neonates were used to obtain bacteria flora samples at various time of day. A variety of microorganisms were isolated from the samples. However, some samples demonstrated no bacterial growth. We observed that neonates during the birth would have colonization with a single bacterial species. Nevertheless.after a period of time several species of bacteria would be intermixed together. Several gram negative bacilli strains isolated at various hours after birth were characterized (Figures 3, 4, 5, 6 and 7).

Oral cavity was found to be sterile in most of times especially during birth and consequently colonization of

Figure 2. Isolate sub cultured.

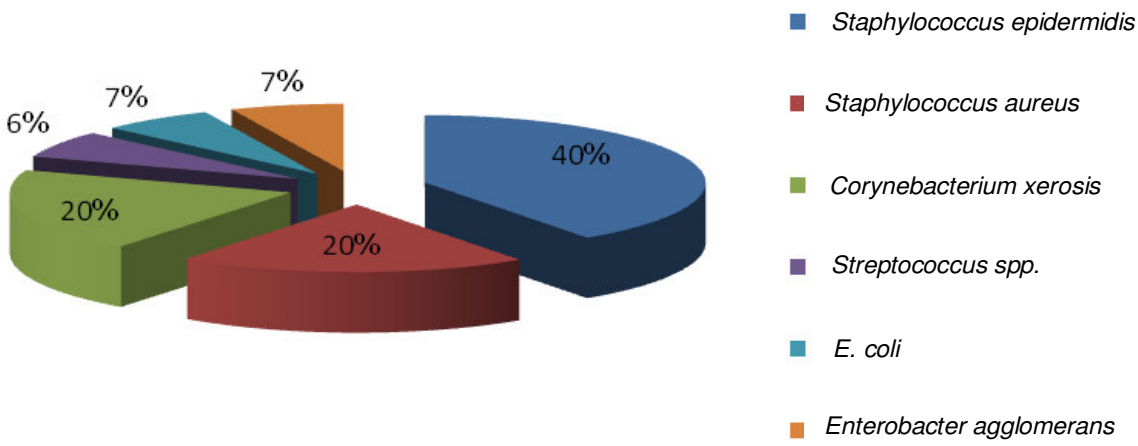

Figure 3. Frequency of isolated organisms from forehead at the birth time.

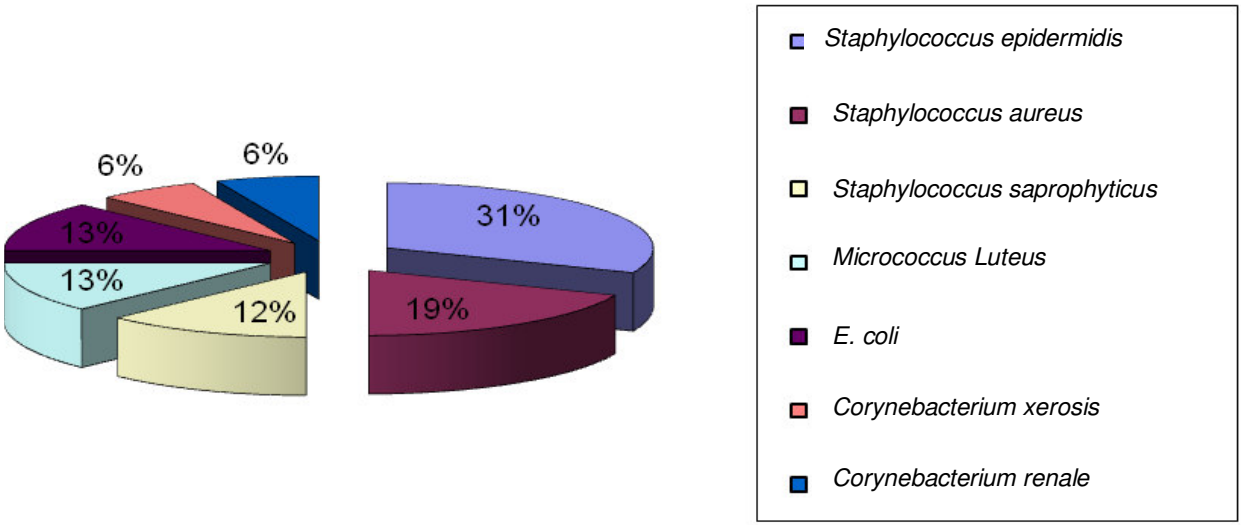

Figure 4. Frequency of isolated organisms from forehead after birth time.

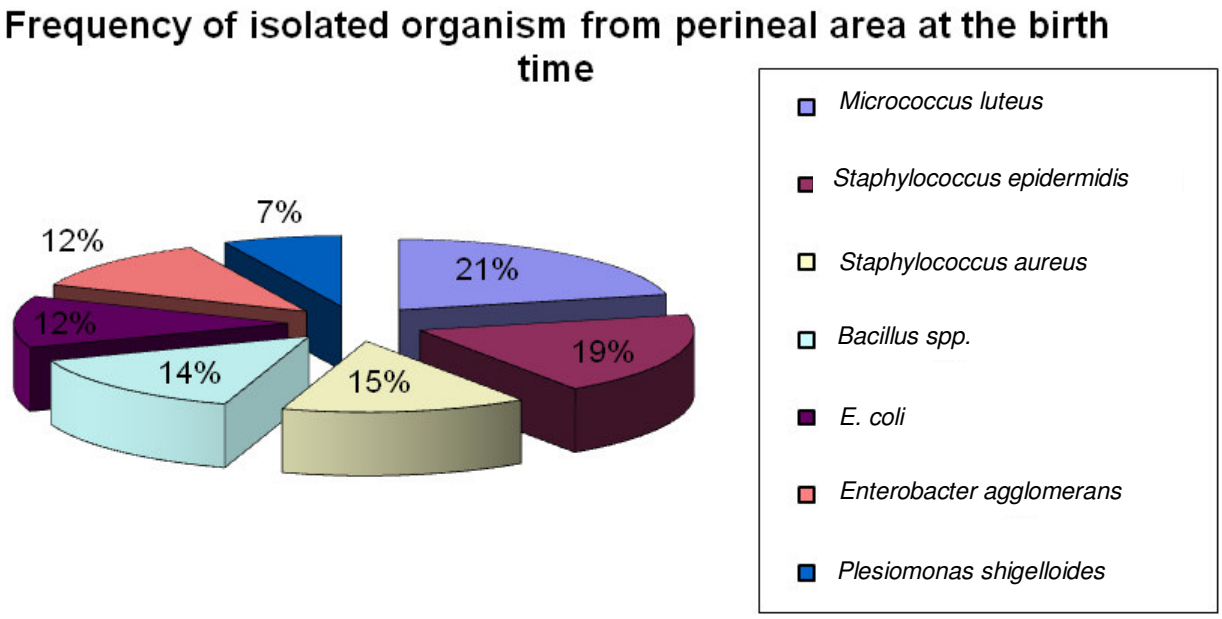

Figure 5. Frequency of isolated organisms from perinea area at the birth time.

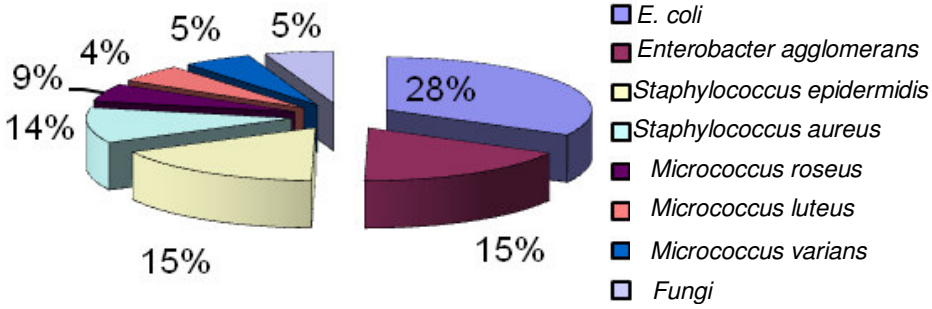

Figure 6. Frequency of isolated organisms from perinea area after birth.

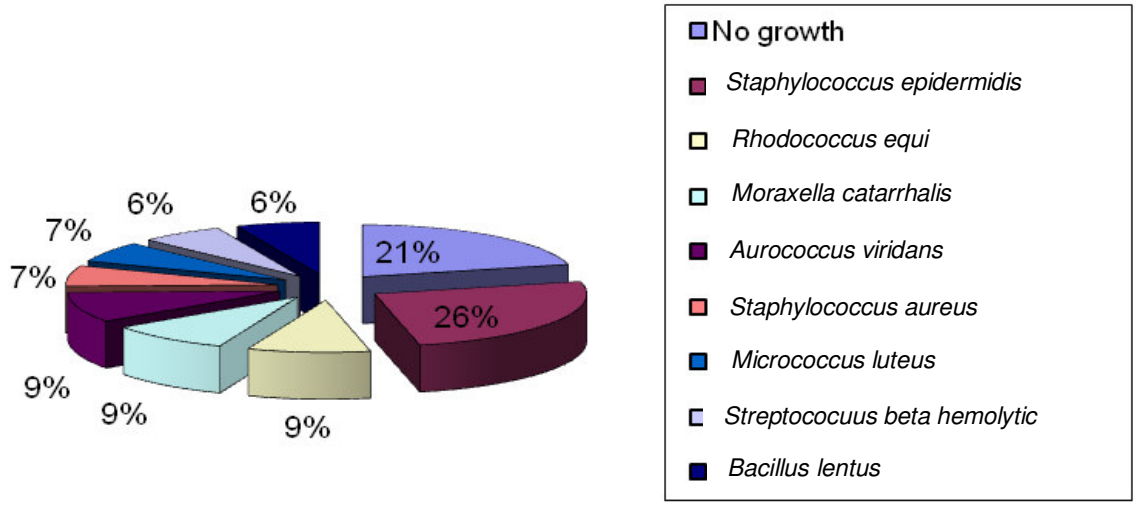

Figure 7. Frequency of isolated organisms from oral cavity 6 h after birth.

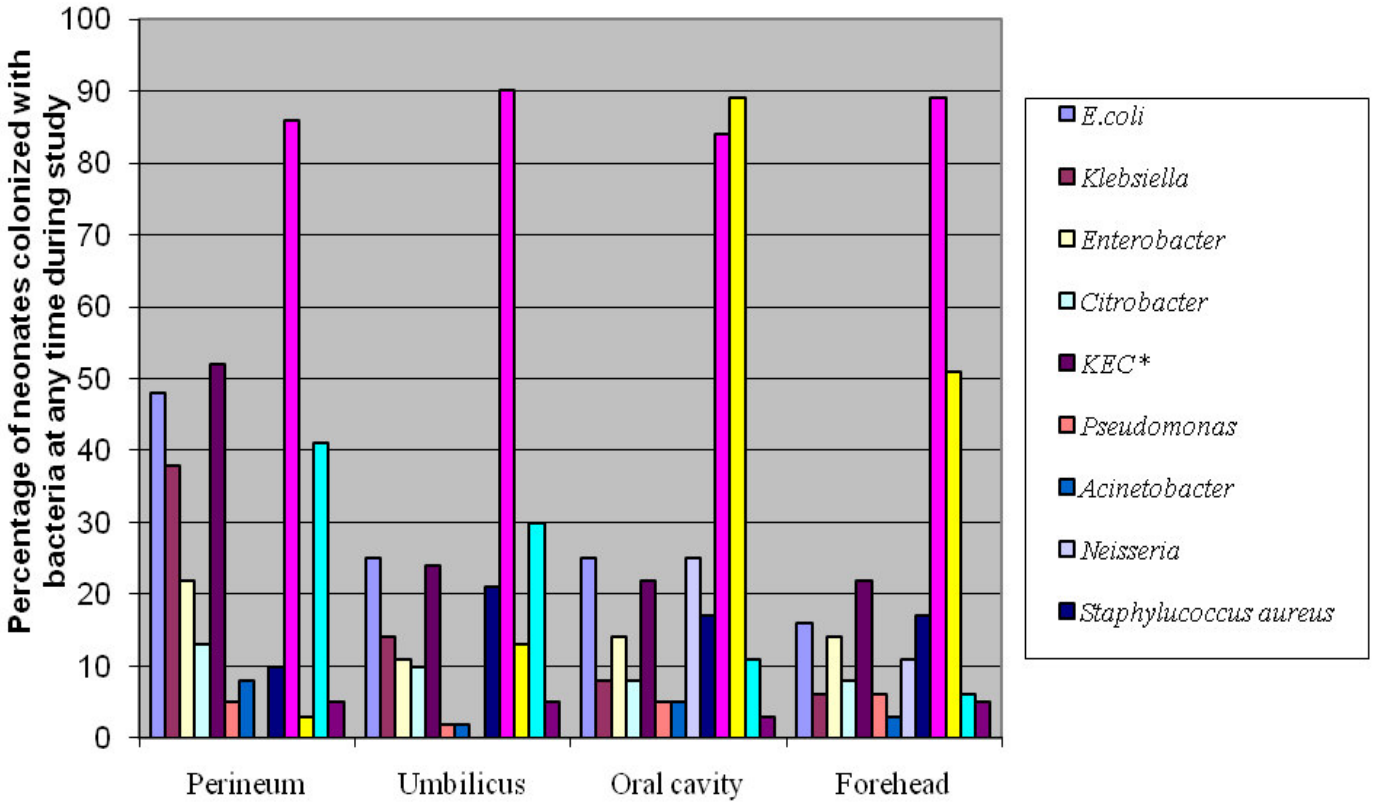

Figure 8. Percentage of infant with organisms in various anatomic sites. * KEC (*Klebsiella, Enterobacter, or Citrobacter*).

bacteria was initiated by ingesting food and other environmental factors (Figure 7).

Although 8 to 15% of cultures yield *β-hemolytic streptococci group B*, which was detected by CAMP and Hippurate hydrolysis test, *group A streptococci* are also encountered. During a two-day period 16 of 22 neonates in one nursery had organisms sensitive to bacitracin and penicillin cultured from their umbilical areas. None of the infant showed clinical signs or symptoms of disease. These organisms were presumptively identified as *group A streptococci* and the positive cultures were first interpreted as evidence of group A *streptococcal* infection. Several weeks later, organisms from 14 of these infants were identified as a single strain of β-hemolytic *Sterptococcus* (Figure 8).

DISCUSSION

Various factors may influence the role of microorganisms in relationship to colonization such as antibacterial agent (bacteriocin), bacterial interference and competitive inhibition. Production of bacteriocin by a bacterial species can inhibit colonization of other bacterial species. It is also known that a floral bacteria growth can be inhibited by another bacterial species through competitive inhibition. For instance pathogenic microorganisms like *Staphylococcus aurous* can proliferate on the neonatal skin in the absence of skin normal flora such as coagulase-negative *Staphylococci*. External factors can also alter the skin normal flora population:

1) The climate: augmentation in humidity and temperature can increase the bacterial growth and hence modifying their population ratio.
2) Anatomical condition, body site: the distribution of skin normal flora on the body depends on different anatomical places. The transient bacteria grow extensively on exposed body parts such as face, neck and hands.
3) Hospitalization, in this study has revealed that long term hospitalization can increases the bacterial colonization in neonates.
4) The disease effect: The presence of systemic disease can also make the body susceptible to bacterial growth. In this study has revealed diabetic neonates born from diabetic mother have greater potential for bacterial growth than the normal neonates.
5) Disinfectant effect: Base on this study organisms such as *Pseudomonas* was the most common, and the *E.coli* and *Entrobacter* were isolated repeatedly from washing liquid. Nonetheless, it was shown that none of these disinfectant washing liquid would change the coagulase-negative *staphylococcus* population, but using disinfecting

soap increased the *Propionobacterium* population extensively.

6) Irradiation: this study show that bacterial growth was susceptible to U.V light radiation and *staphylococci* were the most susceptible one among all the studied organisms.

One of the controversial points in this study was the level of colonization for *group B Streptococci* among the neonates.

According to the study in the Cooper Green Hospital, the rate of colonization for *sterp B* was established during 28 months. They emphasize that *strep B* was the most common cause of infection in neonate and twines in which being considered as high risk group (William et al., 1999). Nevertheless we found variation in the amount of these bacteria in forehead and perinea areas (14.2 and 3.5%).Therefore it can be concluded that the main cause of *strep B* colonization in neonate was due to exposure of birth canal.

Base on reported studied (Falle and Schleifer, 1981). at this study ,bacterial growth were increased in still-birth ,low-weighted birth neonates and also in some twins and single-born babies ,this increase however was more profound in twins due to complex delivery involved. We also have found that there is an increasing rate of colonization of bacteria in immature and low weight neonate.

Some studies showed a significant difference exist in colonization of bacteria on skin of neonates during the first, fourth and tenth weeks. We have demonstrated that although the rate of colonization of bacteria after birth is undetectable but colonization of gram- negative organisms was more prevalent.

Nobel and Jackman reported that colonization of *staphyloccocus* in axillaries area can decrease rate of the *Coryneforms* of bacteria (William et al., 1999).

But we have showed different result and that the rate of colonization of these bacteria was similar both during and after birth.

We also concluded that the highest rate of colonization for various species of *staphyloccocus* was in axillary's areas .and the highest rate of colonization for *Coryneform* bacteria was reported in forehead and auxiliary's areas. The *S. aureus* and S. *epidermidis* were the most common organisms in forehead area.

The most common organisms of external ear were reported to be *S. epidermidis*; we found the same result which confirmed pervious studies.

Some of earlier researches and literatures have reported that anaerobic *Sterptococcus* species are the first cause of infection. It seems that specific species constitute the normal flora of vaginal and oral cavity.

While being born the skin of neonates with cesarean surgery was found sterile during delivery, but neonates born through birth canal was found to be colonized with the normal flora.

Based on other results.the *Satphylococcus* and *Coryneforms* were reported as the most common organisms.found in neonate skin and *E.coli* and *Proteus* species (10%) were also reported. In this study the same result has obtained (Sarkany and Gaylarde, 1967).

The *S. epidermidis* was colonized in upper parts of body and consisted more than 50% the of *Staphylococcus* species. We found the same result too.

The *S. 0hominis* and *S. hemolyticus* were found in auxiliary's and perinea areas. Other findings indicated that coagulase negative *staphyloccocus* was colonized in perinea area. According to other researches, intestine of neonates were colonized with high rates of *klebsiella*, E. *ntrobacter* or *Citobacter* species (William et al., 1999).

We found that the most common organisms in neonate s, stool were gram- negative bacteria such as *E.coli* and *Entrobacter* species.

We also observed that gram -negative bacilli were mostly colonized in neonates care units. Therefore, we disregarded studying bacteria colonization in the first hours of birth in care units.

Larson reported an inverse relation between the bacteria colonization and continuous hand washing of personals and nurses of hospitals.

Moreover, hands can be a transmission bacteria portal between the neonates. Therefore, it is highly recommended, to use disinfectants daily and hand washing should be done in neonates care units and ICU as well (Pass et al., 1980).

Ethical aspects

Informed consent was obtained from the parents of the babies.

REFERENCES

Guenthner S.H, Owen Hendley J, Wenzel RP (1987). Gram-Negative Bacilli as No transient Flora on the Hands of Hospital Personnel. J. Clin. Microbiol., 25: 488-490.

Penders J, Thijis C, Vink C, Stelma FF, Snijders B, Kummeling I, Brandt PAvan den, Stobberingh EE (2006). Factors Influencing the Composition of the Micro biota in Early Infancy. Pediatrics, 118: 511-521.

Falle A, Schleifer KH (1981). Modified Oxidize and Benzidine Tests for Separation of Staphylococci from Micrococccci. J. Clin. Microbiol., 13: 1031-1035.

Pezzati M, Biagioli EC, Martelli E, Gambi B, Biagiotti R, Rubaltelli FF (2002). Umbilical Cord Care The Effect of Eight Different cord-Care regimens on: Cord Separation Time and Other Outcomes. Biol. Neonate, 81: 38-44.

McConnell TP, Lee CW, Couillard M, Westbrook Sherrill W (2004). Trends in Umbilical Cord Care: Scientific Evidence for Practice. Newborn Infant Nurs. Rev., 4: 211-222.

Pass MA, Khare S, Dillon HC (1980).Twin pregnancies: Incidence of group B streptococcal colonization and disease. Brief clinical and laboratory observations. J. Pediatr., 4: 635-637.

Goldmann DA, Leclair J, Macone A (1978). Bacterial colonization of neonates admitted to an intensive care environment. J. Pediatr., 93(2): 288-293.

McGarrity GJ, Coriell LL (1973). Bacterial Contamination of Children's Soap Bubbles. Am. J. Dis. Child., 125: 224-227.
William EB, Jeffrey BG, Maurice LD (1999). Risk Factors for Early-onset Group B Streptococcal Sepsis: Estimation of Odds Ratios by Critical Literature Review. Pediatrics, 103: e77.
Sarkany I, Gaylarde CC (1967). Skin flora of the newborn. Lancet, 1: 589-590.