Survey of Pathogenic Bacteria in Neonatal Intensive Care Unit of Tripoli Medical Center, Libya.

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الملخص

عناية حديثي الولادة من اهم الاماكن التي يرتفع فيها معدل الالتهابات ويتم فيها انتقال البكتريا الممرضة بين المرضى الذين يتلقون العلاج فيها مما يؤدي الى انتشار المقاومة للمضادات الحيوية وزيادة حالات الوفيات بين الاطفال. لمعرفة اشهر البكتريا الممرضة و معدل انتشارها في عناية حديثي الولادة بمستشفى مركز طرابلس الطبي و اماكن تواجدها و مدى استجابتها للمضادات الحيوية و معدل المقاومة لهذه المضادات.

كانت هذه الدراسة وصفية مقطعية. اجريت لحديثي الولادة الذين تم دخولهم في ثلاثة اشهر مايو– يونيو –يوليو من عام 2013 حيث كان عددهم الاجمالي 479 منهم 244 كان دخولهم للعناية المركزة و 235 العناية الخاصة بأخذ العينات اللازمة لأجراء المسح الطبي لمعرفة سبب الالتهاب المصاحب لدخول الطفل للعناية كذلك المزرعة الميكروبية والمضاد الحيوي المناسب للعلاج واذا مصاحب لهذا العلاج أي نوع من المقاومة للعقار.

اظهرت النتائج ان اشهر بكتريا ممرضة في العناية هي اسينوباكتر بوماني و بمعدل (%40– %48) يتبعها الكليبسيلا نمونيا بمعدل (31% – 33%) واشهر اماكن الاصابة هي الاذن والانف و الحلق بنسبة (53 % – 58%) مقارنة بالدول الاخرى ان معدل انتشار العدوى متقارب مع دول العالم.

1. ABSTRACT:

Neonatal intensive care unit (NICU) is an ideal environment for transmission of many pathogens, which lead to high morbidity and mortality in neonates. Microbial pathogens which cause nosocomial infection are known of high resistance to many antibiotics which lead to economic burdens, in addition to social and psychological factors affecting the families due to long duration in hospitals. Prevention of infection requires identification of contaminated environment and equipment, effective infection control programs, and judicious of antimicrobial agents.

Methods: 244 neonates, who were admitted to NICU, and 235residing neonates at Special Care Baby Unit (SCBU) of Tripoli Medical Center (TMC) in the months of May to July 2013, and who were found positive for pathogenic infection were subjected to the study. Pathogenic organisms were isolated and identified according to Standard Microbiological Methods, and antibiogram was assessed against common antibiotics.

Results: Acinetobacter baumannii was the most frequently isolated bacterium from neonates at NICU and SCBU at a rate of 40% and 48%, followed by Klebsiella pneumoniae at a rate of (33% and 31%) respectively, while the less frequently isolated organisms were Staphylococcus haemolyticus (9% NICU and 5% SCBU), and S. hominis (7% NICU and 3% SCBU). Infection sites were 2 Ear, Nose, and Throat (53 % NICU and 58% SCBU), CSF (33% NICU and 3.85% SCBU), Urine, Eye swab (30% NICU and 47% SCBU), Vein Catheter 29 %) NICU), Skin swab catheter (30% NICU and 27% SCBU), blood (11% ICU and 12% SCBU), Umbilical Swab (7% NICU and 18% SCBU), Nasal swab (4% NICU and 12%S CBU), catheter (30.33NICU and 27%S CBU(.

 Conclusion: data indicate significant infection problem in Tripoli Medical Center Hospital, and inefficient infection control programs. A. baumannii and Klebsiella pneumonia were the predominant cause of neonatal infection at TMC. Antimicrobial resistant pathogens isolated in Neonatal Intensive Care and Special Care Baby Units respectively were: K. pneumoniae (ESBL)(56.52% and 44.44%), Carbepenem-resistant K. pneumoniae 21.7%) and 11.11%), and E. coli (ESBL) (13.04% and 5.56%).

2. INTRODUCTION :

At any point of time, over 1.4 million people worldwide are suffering from infections acquired infection at hospitals. Between 5% and 10% of patients admitted to modern hospitals in the developed world acquire one or more infections. The risk in developing countries is 2 to 20 times higher (Pittet D .,and Donaldson 2006). In some developing countries, the proportion can exceed 25%)Mayon et al., 1988). Infections are an important cause of neonatal morbidity and mortality worldwide. Neonatal infections among low-birth weight infants are associated with significant risk of neurologic abnormalities, developmental and functional delays (Vohr et al., 2000(. Although most neonatal infections are of maternal or community origin, an increasing proportion of these infections is acquired in the nursery. (Zafar et al., 2001). Nosocomial bloodstream infections

(BSIs) are increasing in prevalence worldwide and result in significant morbidity, mortality (Rupp M.,2004). In recent years, the subject of the emergence and subsequent increase in the incidence of resistance to antimicrobial agents has become a serious threat)WHO, 1999.(Reports from all around the world suggest that antibiotics are rapidly losing their effectiveness, with some early reports going so far as to suggest that we are approaching a post-antibiotic era (Cohen M., 1992). Antimicrobial resistance is increasing for a variety of reasons, these includes un-optimal use of antimicrobials for prophylaxis and treatment of infection, prolonged hospitalization, increased number and duration of intensive-care-unit stays, multiple co-morbidities in hospitalized patients, increased use of invasive devices and catheters, (Osmon D., 2001).

3. OBJECTIVE :

This study intends to determine the following:

1. Rate of infection and the main source of these infection.
2. Identity of different pathogenic bacteria found in neonatal intensive care unit at Tripoli Medical Center, depicting isolate identity, infection rates, infection sites, anti-microbial susceptibility patterns of isolated bacterial agents.
3. Determine antimicrobial resistance of organisms isolated in the study.

4. LITERATURE REVIEW :

There was study carried out to survey both the epidemiology of nosocomial infection in NICU and the annual trends of pathogens in Korea, which occurred in NICU from January 1995 to December 1999. The data included clinical characteristics, site of infection, pathogens, and mortality. Nosocomial infection rate was 9.0 per 100 NICU admissions during the five-year period. Major sites of infection were bloodstream (32.3%), skin (18.4%), endotracheal tube (17.2%), and catheter (10.6%). The most common pathogen was S. aureus (29.9%). and the others were coagulase-negative staphylococci (CONS) (14.8%), Enterobacter (12.4%), and Candida (9.0%). During the five-year period, nosocomial infection rates increased from 9.5 to 11.6 per 100 admissions with the increase of CONS, Candida, Klebsiella, and Acinetobacter baumannii, while the infection rate of S. aureus decreased. (Kwon H, et al.2002).

There was a case records of all neonates admitted to the NICU of TMC, Libya for the period Sep.1996 through August 1997, inclusive, were reviewed. Blood and/or CSF cultures were used to establish the diagnosis of bacterial infection. The

admissions were categorized as sterile and unsterile. A total of 1123 newborns were admitted to NICU over the period of the study, 129 (11.5%) of them were proved to be bacterially infected, 10.6% and 24% of the sterile and unsterile admissions, respectively, had bacterial infection. Blood culture was positive in 115 (10.2%) of the admitted newborns, while CSF culture was positive in 24 (2.1%) of them. Gram-negative bacteria were the predominantly isolated bacteria. Serratia spp. was isolated from (38.3% and 50%) of blood and CSF cultures, respectively. K. pneumonia was isolated from about (25%) of both blood and CSF cultures. Coagulase negative staphylococcus (CONS) was isolated from (11.3%) of blood cultures. we can conclude from this study that neonatal infection is still a problem facing the country and there is a need for 6 study of bacterial colonization of anogenital tract of Libyan pregnant women and its relation to neonatal infections (Dekna, et al.2007(.

In a Brazilian neonatal intensive care unit (NICU), a study was a retrospective cohort from January to December, 2003. All neonates were admitted to the NICU. Study was conducted at a public, tertiary referral NICU of a teaching hospital in the Northeast of Brazil. A total of 948 medical records were reviewed. Overall NI incidence rate was 34%. (Ana Carolina, et al 2008. (

From February 2004 to January 2005, 579 consecutive episodes of blood stream infection were obtained at two neonatal intensive care units Al Nasser and Al Shifa hospitals in Gaza City. Forty (6.9%) isolates of A. baumannii were obtained from the neonates under 28 d. Most of the isolates (92%) were from hospitalized patients in the intensive care units. Community acquired infection was 8%. Sixty three percent of the patients were males. The isolates of A. baumannii were resistant to commonly used antibiotics while being sensitive to meropenem (92.5%(, imipenem (90%), chloramphenicol (80%), ciprofloxacin (75%), gentamicin (57.5%), ceftriaxone (50%), amikacin (37.5%), cefuroxime and cefotaxime (35%). Over all crude mortality rate was 20% with much higher crude mortality among patients with nosocomial infection. Based on logistic regression, the following factors were statistically significant :weight < 1500g, age < 7 d, mean of hospitalization equal 20 days, antibiotic use, and mechanical ventilation, when compared to the control group (P<0.05) (Aljarosha, et al, 2008(.

A noted study was carried out in September 2011. A three-month descriptive cross-sectional survey in two intensive care units in Gaza city (Al-Shifa and Al-Nasser hospitals). Results concluded that a total of 622 cases showed an incidence

rate of septicemia at 10.4%. The causative bacteria were: coagulase- negative Staphylococcus (39%), S. aureus (23%), Streptococcus spp. (12%), Pseudomonas spp. (8%), and E. coli and K. pneumoniae (5% each). Lack of institutional commitment and worker's interest in preventive measures, shortage of environment disinfection and unfacilitated hand washing are the main negative observations. The environment and healthcare workers were found loaded with potential bacterial pathogens. Ampicillin, Gentamicin and Cefotaxime (claforan) are extensively used in the units as a prophylactic medicine. K. pneumoniae has shown the highest rate of antibiotic resistance (53%)Eljadba, et al. 2009(.

A perspective study was conducted to describe the epidemiologic profile of nosocomial infection in the neonatal intensive care unit (NICU) in china. The newborn infants who were admitted in the NICU for more than 48 hrs. were enrolled from February 2006 to January 2007. The clinical data were collected. The rate of nosocomial infection was calculated according to the CDC (centers of disease control) surveillance system. The risk factors of nosocomial infection were investigated by multivariate regression analysis. A total of 1 159 neonates were recruited. A total of 169 nosocomial infections occurred, with a cumulative rate for nosocomial infection of (14.58%). The incidence of nosocomial infection was 19.52 per 1 000 patient-days. Ninety-two cases of pneumoniae, including 38 cases of ventilator-associated pneumonia (VAP(,were reported, with a nosocomial infection rate of 7.94%, which was the most common nosocomial infection in the NICU. Among these infants the rate of VAP was 48.8 per 1 000 ventilator days. The major microorganisms isolated from the infected patients were A. baumannii, K. pneumonia, and coagulase negative staphylococcus. Birth weight (OR 2.130, 95% CI 1.466-3.094), mechanical ventilation (OR 7.038, 95%CI 3.901-12.698), chest tube drainage (OR 7.004, 95% CI 1.841-26.653) and ibuprofen therapy (OR 2.907, 95% CI 1.303-6.487) (Cai XD, et al. 2010.(

A cohort study which measured the occurrence and risk factors of nosocomial infections in the neonatal intensive care unit was carried out in Abha General Hospital, Saudi Arabia. Of 401 neonates who stayed at least 48 hours in the unit, 77 developed bacterial infections. The most frequent infections were: pneumonia (50.0%), bacteremia (40.9%) and skin and soft tissues (6.5%). In logistic regression analysis, mechanical ventilation and parenteral nutrition were identified as significant risk factors. Neonates suffering from nosocomial infections had more than 3 times the risk of dying compared to neonates free of infection (Mahfouz, et al. 2010).

A prominent study in Egypt was published lately in 2013 with an objective to determine the incidence, anatomical sites and causative organisms of NI in an Egyptian NICU,. This was a descriptive hospital-based study carried out for 12 months in the NICU of the Mansoura University Children's Hospital. NI rates were calculated using different denominators (overall nosocomial infection rate, nosocomial infection incidence density, device-specific infection rates and device-days infection rates). Of the 238 neonates evaluated, 49 developed 51 nosocomial infective episodes, equating to an incidence rate of 21.4% or 13.8 infections per 1000 bed-days. Pneumonia was the most frequently occurring infection (11.3%) followed by bloodstream infection (8.8%). The most frequently isolated organisms were Klebsiella spp. (33.3%) followed by E. coli (21.6%). NIs were associated with prolonged hospital stay(Abdel-Wahab, et al. 2013).

When we compare our study with all previous study there is no significant different and mostly there is similar pattern and infectious agent with our hospitals.

5. SUBJECTS AND METHODS:

Tripoli Medical Center is tertiary care center. The NICU (neonatal intensive care unit) consists of five rooms with capacity of 40 neonates from inside the hospital. SCBU (special care baby unit) with capacity of 30 neonate servers for babies referred from outside the hospital and outside Tripoli. Three months descriptive Cross- sectional study done where the data were collected from newly admitted neonates for more than 24 hour .

during a period of three months (May, June, and July 2013). 479 neonates were examined at the time of admission the following information were recorded, gestational age, birth weight, mode of delivery, exposure vascular catheter, endotracheal tube, mechanical ventilation.

Full septic screen was performed, bacterial isolates were run through Gram stain, and biochemical identification using different culture media were used for different samples, Table1, (Murray, et al 2007), and culture identification tests based on biochemical features according to Standard Microbiological Methods (Winn, et al. 2005).

6. Protocol for specimens processing :

Blood samples were obtained from infant, most samples were collected prior to antimicrobial therapy. Superficial swab (rectal, nose, ear, umbilical, skin, eye) for

every admitted neonate, UVC TIP, endotracheal tube, umbilical vein catheter, urine catheter cerebrospinal were all processed for culturing on appropriate media shown in Table 1. Instrument tip (ENT, Catheter tip, CSF for Gram stain and culture if indicated. Sputum or throat swab, joint fluid aspiration and culture if indicated.Venous blood was obtained from admitted newborns by nursing staff by means of a septic technique: briefly 0.5ml of blood was drawn in to aerobic bottle (broth media) incubated at 37C aerobically and checked daily for turbidity for six days. Turbid bottles were cultured on Blood agar, MacConkey agar, Chocolate agar, and incubated aerobically at cytoperm incubator with 10% Co_2 for 24h at 37C, followed by bacterial isolation and identification.Swabs were used for umbilical, eye, ear, skin and cultured on Blood Agar , MacConkey agar, Chocolate agar, and Thioglycolate broth.CSF samples were cultured on Blood Agar, Chocolate Agar, Brain Heart Infusion Broth (BHI), while Catheter tips were cultured on Blood Agar, MacConkey and BHI. Nasal swabs were cultured in Blood Agar and MacConkey agar. After 24 hr bacterial isolates for were picked for identification and antibiogram testing. Plates or broths with no growth were further incubated for 72 hs. and colonies were processed as indicated. Identification, and antimicrobial resistance for isolated pathogen were determined according to National Committee of Clinical Laboratory Standards , break point value was done with VITEK Susceptible System.

Disc Diffusion Method:

Antibiotic susceptibility testing for isolated bacterial pathogens was done according to Bauer and Kirby (1966) using Disc diffusion method using Mueller-Hinton agar with commercial antibiotic disc discs: amoxicillin, cefotaxime, cefixime, ciprofloxacin, ampicillin, nitrofurantoin, norfloxacin, ofloxacin, amikacin, nalidxic acid, ceftazidime, ceftriaxone and gentamycin. Plates were incubated at 37C for 24h.

7. RESULTS:

NICU and SCBU at Tripoli Medical Center suffer from potential contamination and hazardous infection. Infection rate as related to body weight of les than 2.5 kg was 49% (n119) and 19% (n44) at NICU and SCBU respectively.

- A. baumannii was the most frequently isolated bacterium from neonates at NICU and SCBU at a rate of 40% and 48%, followed by K.pneumoniae at a rate of (33% and 31%) respectively, while the less frequently isolated

organisms were S. haemolyticus (9% NICU and 5% SCBU), and S. hominis (7% NICU and 3% SCBU .(

- Isolated pathogens in NICU and SCBU respectively were:
  o A. baumannii (40% and 48%.(

  o K. pneumoniae (33% and 31%).

  o S. haemolyticus (9% and 5%(.

  o S. Hominis (7% and 3%).

  o Entercoccus spp (7% and 5%.(

  o E. coli (5% and 9%).

Main sources of infection in Neonatal Intensive Care and Special:

Care Baby Units were:

- Endotracheal tubes: 37 samples total, 20 positive (54%).
- Eye swab (C/S): 35 samples total, 13 positive (37%(.
- Umbilical vein catheter: 35 samples total, 10 positive (29%(.
- Urine Catheter: 25 samples total, 7 positive (28%).
- CSF: 41 samples total, 6 positive (15%).
- Umbilical swab: 206 samples total, 25 positive (12%).
- Blood Culture Sensitivity (C/S) 292 samples total, 32 positive (11%.(
- Rectal swab: 210 samples total, 21 positive (10%(.
- Nasal swab: 270 samples total, 18 positive (7%).
- Skin swab: samples total, 10 positive (7%).

Antimicrobial resistant pathogens isolated in Neonatal Intensive Care and Special Care Baby Units respectively were:

- K. pneumoniae (ESBL) (56.52% and 44.44%).
- Carbepenem-resistant K. pneumoniae (21.7% and 11.11%) E. coli (ESBL), (13.04% and 5.56%).
- E. coli (ESBL), (13.04% and 5.56%).

The following Tables show more detailed numbers and percentages of all tested pathogens, antibiotics, and body sites.

Table 1. Isolated pathogens in NICU and SCBU .

Type of organism	NICU		SCBU		X²
	NO	%	NO	%	
Klebsiella pneumoniae	33	32.67	20	3076	
Acinetobacter baumannii	40	39.60	31	47.69	
Staph. haemolyticus	9	8.91	3	4.62	4.18 NS
Staph. hominis	7	6.93	2	3.07	
E. Coli	5	4.95	6	9.23	
Entercoccus spp	7	6.93	3	4.62	
Total	101		65		
Total infection(%)	(101/244) X 100 = 41.39		(65/235) X 100=27.66		

Table 2. Pathogens isolated from blood samples.

Blood Culture Sensitivity (C/S) Type of organism	NICU Total sample(190) Positive sample (20)		SCBU Total sample(102) Positive sample (12)	
	NO	%	NO	%
Klebsiella pneumoniae	5	2.63	5	4.90
Acinetobacter baumannii	6	3.16	5	4.90
Staph. haemolyticus	3	1.58	2	1.96
Staph. hominis	4	2.11	2	1.96
Enterococcus spp	3	1.58	---	---
Strep. a galactiae	1	0.53	---	---
Non hemolytic streptococcus	1	0.53	---	---
Total infection(%)	(20/190) X 100 = 10.53		(12/102) X 100=11.76	

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Table 3. Pathogens isolated from endotracheal samples.

Endo Tracheas tube Type of organism	NICU Total sample(25) Positive sample (13)		SCBU Total sample(12) Positive sample (7)	
	NO	%	NO	%
Acinetobacter baumannii	10	40	6	50.00
Klebseilla pneumoniae	5	20	3	25.00
Staph. haemolyticus	1	4	1	8.33
Pseudomonos aeruginosa	3	12	---	---
Entercoccus coleacea 1	1	4	---	---
Entercoccus faecalis 1	1	4	---	---
Total infection	(13/25) X 100 = 52.00		(7/12) X 100 = 58.33	

Table –4– Biogram of organisms isolated in NICU and SCBU.

Type of organism and drugs resistance	NICU		SCBU	
	NO	%	NO	%
MRSA	2	8.70	7	38.89
Klebsiella pneumoniae (ESBL)	13	56.52	8	44.44
E. Coli (ESBL)	3	13.04	1	5.56
KP Carbapenem. resistance	5	21.74	2	11.11

8. DISCUSSION:

Babies are born without an endogenous microbial flora and rapidly become colonized with microbes encountered in the maternal genital tract and their immediate postnatal environment. Since their immature immune system is unable to provide a robust defense against virulent pathogens, neonates are at high risk of developing invasive infections if exposed to pathogenic microorganisms. Premature neonates are at especially high risk of infection because of their lack of protective maternal antibodies, underdeveloped innate immunity and their fragile, easily damaged skin.

Our study showed a relatively high infection rate in TMC (NICU and SCBU) but comparing it with other hospitals in other countries we realize that our local situation is not an extreme. Rates of infection were 41 and 28 % in Tripoli NICU and SCBU, respectively should not be regarded as average by all means. Such high numbers call for prudent and immediate attention for maximum infection control measures, especially that we now know the aetiological agents behind this dilemma. Significant pathogens isolated from NICU and SCBU respectively were A. baumannii (39.60% and 47.69%), and K. pneumonia 32.67%) and 30.76%). These pathogens commonly cause similar problems in other countries as well (Eljadba et al, 2010), basically denoting gross negligence and lack of hygiene and infections control programs.

9. RECOMMENDATIONS:

Simply, improving hand hygiene. Soap and sinks should be accessible. Alcohol- based rubs should be available at the entrance to the infant's room. Written procedures that encourage routine hand washing should exist. The use of antibiotics in the NICU should be carefully monitored. broad-spectrum antibiotics should be used until culture and sensitivity results are available.. An isolation room should be available for NICU patients. Furthermore, adequate staffing is more critical in special care units, where virulent pathogens and immunocompromised patients are more common the women bearing, Initiation of interal feeding as early as possible and promotion of the use the human milk.

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