Common pathogens associated with neonatal bacteremia and their antibiotic resistance pattern.

Abdulbaset. M.E. Abusetta[1] M. A. B.Gamal[2] and Fathia A. Ben saed[3]

1 Pathology Department, Faculty of Medical Technology, Tripoli University, Tripoli, Libya.
2.Microbiology Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.
3.Tripoli Health Care ,Ministry of Health, Libya
abustta@yahoo.com

Abstract: Background: Despite recent advances in prenatal care, neonatal sepsis remains a major cause of morbidity and mortality. Neonatal bacteremia occurring in neonates older than 3 days occurs in approximately 10% of all neonates and in >25% of very low birth weight infants (≤1500 g) who are hospitalized in neonatal intensive care units**. Objective:** To determine the incidence and evaluate the antimicrobial-susceptibility patterns of bacterial infections in our neonatal units. **Materials:** This study was conducted at Neonatal department, at medical Tripoli center, Tripoli. During the period from march 2010 to August , 2010. In this study 150 infants suffering from neonatal bacteremia were included in the current study. **Methods:** Blood culture, was done using an automated continuous-monitoring blood culture system. The prevalence and antibiotic resistance patterns of bacterial strains were studied. **Results:** It was clear from the present results that the rate of infection decreased with increasing birth weight. Similarly, the infection rate was inversely related to gestational age. There were nine different identified bacterial species (five Gram negative and four Gram positive organisms) and one yeast. The most common Gram positive organism causing neonatal bacterimia in an descending order, were as follow; *Staphylococcus epidermidis* (72.0 cases), *Staphylococcus aureus*, *Enterococcus spp*, *and Streptococcus pyogenes* (isolated from 14.0, 6.0 and 4.0 cases respectively). However, Gram-negative organisms causing neonatal bacterimia in an descending order, were as follow; *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosae*, *Enterobacter*, and *Serratia* were isolated from 15.0, 10.0, 5.0, 3.0 and 3.0 cases respectively. *Candida albicans* was isolated from 18 cases in this study. Most bacterial isolates including *Pseudomonas aeruginosae*, were susceptible to Amikacin. Antibiotic agents whose effectiveness was comparable to Amikacin included Ofloxacin, and Augmenten. Generally, there was a trend of increasing resistance to commonly used antibiotics. **Conclusions:** The trend of increasing bacterial resistance to commonly used antibiotics necessitates the implementation of a rational empirical treatment strategy, based on local susceptibility data, reserving certain agents for emerging resistant pathogens.
[Abdulbaset. M.E. Abusetta M. A. B.Gamal and Fathia A. Ben saed. **Common pathogens associated with neonatal bacteremia and their antibiotic resistance pattern.** *Nat Sci* 2014;12(2):122-126]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 18

Keywords : Neonatal , Bacterial infection, Antibiotic resistance

Introduction:

Despite recent advances in per natal care, neonatal bacteremia remains a major cause of morbidity and mortality. Increased utilization of long-term indwelling catheters and other invasive procedures and the administration of parenteral alimentation were followed by the growing prevalence of nosocomial infections caused by different bacteria [(1)]. Neonatal bacteremia occurring in neonates older than 3 days occurs in approximately 10% of all neonates and in >25% of very low birth weight infants (≤1500 g) who are hospitalized in neonatal intensive care units [(2)]. Central venous catheters are a cornerstone of neonatal intensive care, unfortunately, catheter-related bacteremia is a frequent life-threatening complication [(3)]. Accurate clinical diagnosis of neonatal bacteremia is often difficult, as signs and symptoms in the neonate may be subtle or vague [(4)]. Although the incidence of bacteremia is relatively low (one to eight cases/1,000 live births), the risk of mortality is quite high, ranging from 10 to 50% [(5)]. Currently, blood culturing is considered to be the gold standard for diagnosing neonatal bacteremia. In fact, it was found that over half of the newborn infants demonstrated bacteremia with less than 10 CFU/ml [(3)]. In critically ill neonates, septicemic infection is generally associated with an increased risk of death and a greater length of hospital stay. Outcome can be improved if prompt and appropriate antibiotic therapy is administered [(6)].

Materials:

1. Neonates: This study was conducted at Neonatal department, at Medical Tripoli center, Tripoli. During the period from March 2010 to August, 2010. In this study 150 infants suffering from neonatal bacteremia were included in the current study. Out of 150 neonates; seventy three infants with

a birth weight (>1000 <1500 g), sixty five infants with a birth weight (>750 to <1000 g), and twelve infants with a birth weight ≤750 g.

2. Blood samples: 1.5 ml obtained from initial central catheter access via umbilical vessels by the help of specialized pediatrician.

3. Blood culture system: an automated continuous-monitoring blood culture system, BACTEC 9050 (Becton Dickinson, Sparks, Md.).

4. Other culture media including; sheep's blood, chocolate, Sabauraud dextrose, Muller Hinton and MacConkey agars, supplied by Oxoid limited, UK.

5. Antibiotic discs supplied by Oxoid limited, UK.

Method:

1. Blood culture: was done using an automated continuous-monitoring blood culture system, BACTEC 9050 (Becton Dickinson, Sparks, Md.)., using a fluorescent sensor for detecting microorganisms and relies primarily on the detection of CO_2 produced by actively metabolizing microorganisms. Between 0.5 and 1.0 ml of whole blood was added to the pediatric-sample-sized, resin-containing blood culture bottles (Peds Plus; Becton Dickinson). The bottles were incubated immediately upon receipt in the microbiology laboratory in accordance with the manufacturer's recommendation[7].

2. Identification of microbial species: All positive vials were subjected to Gram-staining and sub cultured for full organism identification on sheep's blood, chocolate, and MacConkey agars.

3. Antibiotic susceptibility testing:

Bacterial susceptibility to antibiotics was conducted by a modified Kirby- Bauer disc susceptibility method (BBL Sensi-Disc; Becton-Dickinson Microbiology Systems, Cockeysville, MD) following overnight incubation on Muller-Hinton agar plates containing 50% blood without added salt at 32ºC to 34 ºC.

Results:

In the present study, maternal demographic data and clinical histories were reviewed , there were no statistically significant associations between infection and maternal age, marital status, prenatal care, multiple gestation, hypertension or preeclampsia, duration of rupture of membranes, mode of delivery, or antenatal antibiotic or steroid therapy. However, birth weight and gestational age were strongly associated with risk of neonatal bacteremia.

Table (1), showed the distribution of infants according to their birth weight. Out of 150 investigated infants suffering from neonatal bacteremia, 73 infants (48.7%) with a birth weight ≤ 750 g, 65 infants (43.3%) with a birth weight > 750 and <1000 g, and 12 infants (8.0%) with a birth weight >1000 and <1500 g. It was clear from the present results that the rate of infection decreased with increasing birth weight. Similarly, the infection rate was inversely related to gestational age; where 81.0 (54%) of the neonatal bacteremia was detected at <31 weeks. The rate of infection declined to 32% at > 31 and <36 weeks, and 14% at >36 weeks, table (2).

Distribution of pathogens isolated from infants involved in the current study are presented in table (3). The vast majority of infections were caused by Gram-positive organisms (isolated from 96.0 cases). Out of those 96.0 cases; *Staphylococcus epidermidis* was the most common pathogen (72.0 cases). Other Gram-positive organisms were *Staphylococcus aureus*, *Enterococcus spp*, *and Streptococcus progenies* (isolated from 14.0, 6.0 and 4.0 cases respectively). Gram-negative pathogens isolated from 36.0 cases of neonatal bacterimia. *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosae*, *Enterobacter*, and *Serratia* were isolated from 15.0, 10.0, 5.0, 3.0 and 3.0 cases respectively. *Candida albicans* was isolated from 18 cases in this study.

The effect of duration of ventilator support on increasing the risk of infection in the studied cases was presented in table (4). The results indicated that; 42% of the infants who were ventilated for 22 - <28 days developed neonatal bacteremia versus only 8% of those who were ventilated for 1 week or less.

Resistances of the isolated organisms to different antibiotics, were investigated and the results were illustrated in tables (5) and (6).

The resistance of gram-positive organisms to the tested antibiotics is presented in tables (5); 100% of *Enterobacter spp.*were resistant to Cotrimoxazole and Rifampicin, 83% were resistant to Gentamicin, 66.6% were resistant to ampicillin, Cefuroxim, Augmentin and Fusidic acid, 50% were resistant to Amikicin. However, all *Enterobacter spp.*isolates (100%) were sensitive to Ofloxacin. The percentages of resistance of *Staphylococcus epidermidis* to the tested antibiotics were as follow; 80% for Ampicilln, Rifampicin and Cotrimoxazole, 30.6% for Cefuroxim and Amikicin and 5.6% for Augmentin, Fucidic acid and Gentamicin. Like *Enterobacter spp.*isolates, all *Staphylococcus epidermidis* isolates were sensitive to Ofloxacin. Concerning the resistance pattern of *Staphylococcus aureus* indicated that high resistance observed with Fusidic acid and Cotrimoxazole (92.9%), followed by Ampicilln, Rifampicin and Cefuroxim (85.7%). Lower resistance was observed with Gentamicin and Amikicin (28.6%). However, all

Staphylococcus aureus isolates were sensitive to both Ofloxacin and Augmentin.

The resistance of gram-negative organisms to the most relevant antibiotics is depicted in tables (6). All *E. coli* isolates were sensitive to Augmentin, Gentamicin, Ofloxacin and Amikicin. Meanwhile 33.3% of *E. coli* isolates were resistant to Ampicilln, Rifampicin, Fucidic acid and Cefuroxim. Higher resistance (73.3%) was noticed with Cotrimoxazole.

Klebsiella pneumonia isolates showed higher sensitivities (100%) to Ampicilln, Augmentin, Gentamicin, Ofloxacin, Rifampicin, Cefuroxim and Amikicin and only 30% of *Klebsiella pneumonia* isolates were resistant to Fusidic acid and Cotrimoxazole. On contrast to *Klebsiella pneumonia* the resistance of *Pseudomonas aeruginosae* (5.0 cases) to Ampicilln, Rifampicin, Fusidic acid and Cotrimoxazole was 100%; while resistance to Gentamicin, Cefuroxim and Augmentin was 60%. However all *Pseudomonas aeruginosae* isolates were sensitive to Amikacin and Ofloxacin. The resistance pattern of *Enterobcter* and *Proteus* spp. to the selected antimicrobial agents were similar, where both species were 100% sensitive to Gentamicin, Cefuroxim, Augmentin, Amikacin, Ofloxacin, Ampicilln and Rifampicin, while 100% of the isolates were resistant to Cotrimoxazole and Fusidic acid.

Discussion:

Despite recent advances in prenatal care, neonatal sepsis remains a major cause of morbidity and mortality. The following data were recorded for all cases; maternal age, marital status, prenatal care, multiple gestation, hypertension or preeclampsia, duration of rupture of membranes, mode of delivery, antenatal antibiotic, steroid therapy, clinical histories, birth weight, age at the time of infection, type of catheter, and ventilator status.

Automated blood culturing systems are good given time that would rule out bacterial septicemia in less time than manual blood culturing [7].

The finding in this study that *Staphylococcus epidermis* is the most common organisms (72) associated with neonatal bacteremia come in agreement with many other studies [4 & 6]. It remains difficult to determine which blood culture isolates of *Staphylococcus epidermis* reflect true infections and which are contaminants. Alternatively, this organism-even if it invades the bloodstream may be less virulent. The majority of *Staphylococcus epidermis* are resistant to the routine antibiotics used to treat newborn infants, and Vancomycin is often required for adequate therapy. It is alarming that at least 48% of infants in this study (whether or not they had documented *Staphylococcus epidermis* infection) were treated with Vancomycin. The Centers for Disease Control and Prevention and others [4], have recommended avoiding empiric Vancomycin therapy in patients with suspected bacteremia to prevent the emergence and spread of Vancomycin resistant strains.

The list of microorganisms isolated from this population of patients more accurately reflects the current spectrum of bacteria and yeast causing infections, both early-onset and nosocomial acquired, in newborn infants than do earlier studies [8& 3]. In the reports of other authors, only 61/201 and 41/175 cultures grew *Staphylococcus epidermidis* and no yeast were cultured in either report [9 & 10]. In 2002, Kicklighter reported 27/98 cultures of *Staphylococcus epidermidis* and 9/98 cultures of yeast in their population of term and preterm infants [9]. That study reflects the trend of the survival of more immature infants and their increased risk for infections with yeast and *Staphylococcus epidermidis*. Our results yielded (18) cultures of yeast and (72) cultures of *Staphylococcus epidermidis*. Yet our rate of positivity at 24 and 48 hours is notably better (46% and 91%) compared with that in the report by Kicklighter 2002 (48% and 79%) when all cultures are considered (cultures growing pathogens, possible-pathogens, yeast, and contaminants) [8]. Our data reflect that the newer technology with improved media is faster in identifying a positive culture even with the inclusion of a high proportion of slow growing microorganisms (*Staphylococcus epidermidis* and yeast) as well as cultures obtained after antimicrobials had been started[11]. The prevalences of organisms causing neonatal sepsis in our population are generally comparable to those previously reported [12 & 13]. The American Academy of Pediatrics recommends the combination of Ampicillin and Gentamicin for the initial treatment of neonatal sepsis and meningitis [14]. Our results demonstrate the pattern of bacterial susceptibility to antibiotics and therefore such cases, if present, are pertinent for the study. Most bacterial isolates including *Pseudomonas aeruginosae*, were susceptible to Amikacin. Thus, the efficacy of the latter agents appears dubious for the empirical treatment of infections in our neonatal units. Of note, other studies found no resistance to these agents in similar sets [13]. Antibiotic agents whose effectiveness was comparable to Amikacin included Ofloxacin, and Augmenten. Because quinolones have been implicated as interfering with cartilage growth in animals, therefore this drug should be used with caution and only in the absence of other options.

Table (1): Distribution of infants according to their birth weight.

Birth weight (g)	Number of cases
≤ 750	73.0
751- <1000	65.0
1001- ≤ 1500	8.0

Table (2): Distribution of infants according to gestational age.

Gestational age (weeks)	Number of cases
>29-31	81.0
>31- 36	48.0
>36	21.0

Table (3): Distribution of bacterial and yeast species associated with neonatal bacteremia

Bacterial species	Number of isolates
Staphylococcus epidermidis	72.0
Staphylococcus aureus	14.0
Enterococcus spp.	6.0
Streptococcus pyogenes	4.0
E. coli	15.0
Klebsiella pneumoniae	10.0
Pseudomonas aeruginosa	5.0
Enterobacter spp.	3.0
Proteus spp.	3.0
Candida albicans	18

Table (4): Effect of duration of ventilation on increasing neonatal bacteremia in studied cases.

Duration (days)	%
<7	8
8 - <14	20
15 - <21	28
22 - <28	42

Table (5): Resistance pattern of the isolated Gram positive organisms to different antibiotics.

Antibiotic	Resistant microorganisms							
	Enterococcus spp.		*Staphylococcus epidermidis*		*Staphylococcus aureus*		*Streptococcus* pyogenes	
	No.	%	No.	%	No.	%	No.	%
Ampicillin	4	66.6	58	80.6	12	85.7	0	4
Cefuroxime	4	66.6	22	30.6	12	85.7	0	4
Amikacin	3	3	22	30.6	4	28.6	0	4
Augmentin	4	66.6	4	5.6	0	0	0	4
Gentamicin	5	83.3	4	5.6	4	28.6	0	4
Ofloxacin	0	0	0	0	0	0	0	4
Cotrimoxazole	6	100	58	80.6	13	92.9	4	0
Fusidic acid	4	66.6	4	5.6	13	92.9	4	0
Rifampicin	6	100	58	80.6	12	85.7	0	1

%= was correlated to the total number of each bacterial species.

Table (6): Resistance pattern of the isolated Gram negative organisms to different antibiotics.

Antibiotic	Resistant microorganisms									
	E. coli		*Klebsiella pneumoniae*		*Pseudomonas aeruginosa*		*Enterobacter spp.*		*Proteus spp.*	
	No.	%	No.	%	No.	%	No.	%	No.	%
Ampicillin	5	33.3	0	0	5	100	0	0	0	0
Cefuroxime	5	33.3	0	0	3	60	0	0	0	0
Amikacin	0	0	0	0	0	0	0	0	0	0
Augmentin	0	0	0	0	3	60	0	0	0	0
Gentamicin	0	0	0	0	3	60	0	0	0	0
Ofloxacin	0	0	0	0	5	100	0	0	0	0
Cotrimoxazole	11	73.3	3	30	5	100	3	100	3	100
Fusidic acid	5	33.3	3	30	5	100	3	100	3	100
Rifampicin	5	33.3	0	0	5	100	0	0	0	0

%= was correlated to the total number of each bacterial species.

References:

1. **Gladstone I. M., Ehrenkranz R. A., Edberg S. C., and Baltimore R. S. (1990):** A ten-year review of neonatal sepsis and comparison with the previous fifty-year experience. *Pediatr Infect Dis J*; 9:819-825.
2. **Merrill, J.D.; Ballard, R.A.; Cnaan, A.; Hibbs, A.M.; Godinez, R.I.; Godinez, M.H.; Truog, W.E. and Ballard, P.L. (2004):** Dysfunction of Pulmonary Surfactant in Chronically Ventilated Premature Infants. Pediatr. Res.; 56(6): 918 - 926.
3. **Benjamin, Jr.; Poole, C.; Steinbach, W.J.; Rowen, J.L. and Walsh, T.J. (2003):** Neonatal Candidemia and End-Organ Damage: A Critical Appraisal of the Literature Using Meta-analytic Techniques. *Pediatrics,; 112(3): 634 – 640.*
4. **Polin, R.A. and Saiman, L. (2003):** Nosocomial Infections in the Neonatal Intensive Care Unit. *Neo Reviews; 4(3): e81 – 89.*
5. **Harbarth S.; Garbino J.; Pugin J.; Romand J. A.; Lew D. and Pittet D.(2003):** Inappropriate initial antimicrobial therapy and its effect on survival in a clinical trial of immunomodulating therapy for severe sepsis. *Am J Med; 115:529-535.*
6. **Kaufman, D. and Fairchild, K.D. (2004):** Clinical Microbiology of Bacterial and Fungal Sepsis in Very-Low-Birth Weight Infants. *Clin. Microbiol. Rev.; 17(3): 638 - 680.*
7. **McGuire, W.; Clerihew, L. and Fowlie, P.W. (2004):** Infection in the preterm infant. *BMJ; 329(7477): 1277 - 1280.*
8. **Kicklighter, S.D. (2002):** Antifungal Agents and Fungal Prophylaxis in the Neonate. *NeoReviews; 3(12): e249 - 255.*
9. **Healy, C.M.; Palazzi, D.L.; Edwards, M.S.; Campbell, J.R. and Baker, C.J. (2004):** Features of Invasive Staphylococcal Disease in Neonates. *Pediatrics; 114(4): 953 - 961.*
10. **Klingenberg, C.; Sundsfjord, A.; Ronnestad, A.; Mikalsen, J.; Gaustad, P. and Flaegstad, (2004):** Phenotypic and genotypic aminoglycoside resistance in blood culture isolates of coagulase-negative staphylococci from a single neonatal intensive care unit, 1989-2000. *J. Antimicrob. Chemother.; 54(5): 889 - 896.*
11. **Krediet, T.G.; Mascini, E.M.; van Rooi, J.; Vlooswijk, J.; Paauw, A.; Gerards, L.J. and Fleer, A. (2004):** Molecular Epidemiology of Coagulase-Negative Staphylococci Causing Sepsis in a Neonatal Intensive Care Unit over an 11-Year Period. *J. Clin. Microbiol.; 42(3): 992 - 995.*
12. **Yu V. Y. (1990):** Neonatal sepsis and infection control policies in Australia. *J. Paediatr Child Health*;26:252-256.
13. **Cordero L., Sananes M. and Ayers L.W. (1999):** Bloodstream infections in a neonatal intensive-care unit: 12 years' experience with an antibiotic control program. *Infect Control Hosp Epidemiol*;20:242-246.
14. **American Academy of Pediatrics(1997):** *Escherichia coli* and other gram-negative bacilli. In: Peter G, ed. *Red Book: Report of the Committee on Infectious Diseases.* 24th ed. Elk Grove Village, IL: American Academy of Pediatrics; 202-204.
15. **Christ W., Lehnert T. and Ulbrich B. (1988):** Specific toxicologic aspects of the quinolones. *Rev Infect Dis*;10:141-146.

2/17/2014