Identification of bacterial agentsand antimicrobial susceptibility of neonatal sepsis with patient's outcome

Abdul-Kareem Mohammed Ali*, Lamia Abdul-Kareem*and Emad Japur Rashed*

الخلاصه

العفن الولادي هو سبب مهم من أسباب الوفيات للأطفال حديثي الولاده.أن نسبة حدوث العفن الولادي البكتيري تعتمد على المنطقه الجغرافيه وقد تختلف من بلد ألى بلد وأيظا في البلد الواحد.

الهدف من الدراسه لمعرفة نسبة حدوث العفن الولادي ، البكتيريا المسببه للعفن الولادي المبكر والمسببه للعفن الولادي المتأخر وأيضا معرفة مدى أستجابتها للمظادات الحيويه ومعرفة نسبة حدوث الوفيات الناتجه من العفن الولادي في وحدة حديثي الولاده.

لقد تم جمع المرضى من وحده حديثي الولاده في مستشفى الكاظميه التعليمي للفتره من الاول من كانون الثاني الى نهاية تشرين الاول لسنة 2011 م. كل المرضى الدين أدخلوا الى وحدة حديثي الولاده والذين لديهم العلامات والاعراض التي تدل على العفن الولادي والتي تم تأكيدها بواسطة زرع الدم الموجب تم أدراجها في هده الدراسه. المعلومات التي جمعت تشمل:عمر الجنين عند الولاده ، الوزن عند الولاده ، جنس المولود ، تأريخ حدوث العفن الولادي ، مكان الولاده ، وكدلك تم متابعة المرضى وتسجيل النتيجه النهائيه للمرض.

في هذه الدراسه ومن 664 مريض تم أدخالهم، كانت نتيجة زرع الدم موجبه ل105 حاله (15,8%). البكتيريا السالبه لصبغة الكرام كانت اكثر انواع البكتيريا المسببه لكل من العفن الولادي المبكر(66,7%) والعفن الولادي المتأخر(56,9%). أن من بين هؤلاء المرضى كانت هناك 33 حاله (31,4%) من عفن الدم المبكر و 72 حاله (68,6%) من عفن الدم المتاخر. أن استجابة البكتيريا للمضادات الحيويه التي جربت كانت متشابهه في حالتي العفن المبكر والعفن المتأخر في هذه الدراسه. أن أكثر من 70% من البكتيريا السالبه لصبغة الكرام كانت مقاومه لكل من الامبسيلين والكلوكساسيلين ولكن اظهرت استجابات متفاوته لكل من الجينتامايسين والسيفوتاكسايم. أن معظم البكتيريا الموجبه لصبغة الكرام اظهرت استجابه لكل من الامبيسيلين والكلوكساسيلين والسيفوتاكسايم بينما اظهرت مقاومه عاليه للجينتامايسين. أن نسبة الوفيات كانت 20,9%. أن العفن المبكر، الذكور، الولاده المبكره الاقل من 37 اسبوع و الوزن الاقل من 2500غرام عند الولاده كانت عوامل مصاحبه للوفاة .

البكتيريا السالبه لصبغة الكرام كانت السبب الرئيسي للعفن الولادي المبكر والمتاخر في مركزنا وان العديد من البكتيريا المسببه كانت مقاومه للمظادات الحيويه المستخدمه.

نوصي بالعنايه الجيده خلال اللحظات الاولى للولاده والمتابعه الافضل اثناء التداخلات الولاديه والكشف المبكر والعلاج اللازم للأم المصابه بالألتهابات مع تقليل التداخلات الجراحيه المساعده للولاده قدر المستطاع مع مراعاة ترك مسافات مناسبه بين الاطفال الحديثي الولادة واستخدام الادوات المعقمه عند المراقبه.

*Dept of Pediatric / Collage of Medicine University AL-Nahrain
*CABP
*M.B.Ch.B

Abstract

Background : Sepsis neonataroum is an important factor for morbidity and mortality in neonates. The incidence of neonatal bacterial sepsis depends on geographic area and may vary from country to country as well as within the same country.

Objective: To identify the percentage of neonatal septicemia confirmed by positive blood cultures among 664 neonates admitted in neonatal care unit, and to identify the bacterial agents causing early and late neonatal sepsis and their antimicrobial susceptibility, and the outcome from neonatal septicemia.

Patients and methods: The total number of patients(with clinical signs and symptoms suggesting sepsis) collected from neonatal care unit of AL-Kadimiya Teaching hospital from the 1st of January to the end of october 2011 were 664 neonates, and only 105 neonates who show signs and symptoms suggestive of septicemia that were confirmed by a positive blood culture were enrolled in this study. Data were collected include :Gestational age, Birth weight , Gender, Onset of sepsis, Place of delivery and also we followed up the subjects and recorded the outcome till discharge.

Results*:* In this prospective study and from 664 neonate were admitted (total number of admission), positive blood cultures were obtained for 105 neonates (15.8%). Gram negative bacteria were the commonest causative agent in both early (66.7%) and late (56.9%) onset sepsis. Among neonates with sepsis, 33 patients (31.4%) had early onset and 72 patient (68.6%) had late-onset neonatal sepsis. The susceptibility of the isolated causative agent to selected antibiotics were the same in early and late onset sepsis. Over 70% of gram negative bacilli were resistant to both ampicillin and cloxacillin but show variable sensitivity to gentamicine and cefotaxime. Most of the isolated gram positive bacteria were sensitive to ampicillin, cloxacillin and cefotaxime but highly resistant to gentamicin. The death rate was 20.9%. Early onset sepsis, male gender, gestational age less than 37 weeks and birth weight less than 2500 gm were found to be significantly associated with death.

Conclusions: Gram negative bacteria were the main cause of early and late-onset neonatal sepsis in our center and many of these isolated bacteria were resistant to the used antibiotics. Low birth weight neonates <2500 gm, gestational age < 37weeks, male gender and early onset sepsis were significantly associated with death. The death rate due to neonatal sepsis was higher compared with the other studies.

Recommendations*:* Proper antenatal care and optimal obstetric management in early detection and treatment of mothers at risks together with minimizing invasive procedures of infants as much as possible and ideal nursery setup which includes adequate space for care of infants and aseptic equipments for monitoring.

Keywords: Neonatal sepsis , neonates , gram negative bacteria , antibiotic resistance , death rate.

Introduction

Infections are frequent and important cause of morbidity and mortality in the neonatal period. As many as 2% of fetuses are infected in utero, and up to 10% of infants have infections in the 1st mo of life. Neonatal infections are unique for several reasons (1). Infectious agents can be transmitted from the mother to the fetus or newborn infant by diverse modes (2). Newborn infants are less capable of responding to infection because of one or more immunologic deficiencies (3). Coexisting conditions often complicate the diagnosis and management of neonatal infections (4). The clinical manifestations of newborn infections vary and include subclinical infection, mild to severe manifestations of focal or systemic infection, and rarely, congenital malformations resulting from infection in the 1st trimester. The timing of exposure, inoculum size, immune status, and virulence of the etiologic agent influence the expression of disease in a fetus or newborn infant (5). Maternal infection that is the source of transplacental fetal infection is often undiagnosed during pregnancy because the mother was either asymptomatic or had nonspecific signs and symptoms at the time of acute infection (6). A wide variety of etiologic agents infect the newborn, including bacteria, viruses, fungi, protozoa, and mycoplasma (7).

The aim of the study:

To identify the culture positive neonatal sepsis among neonates presented with signs and symptoms suggestive of neonatal sepsis in the nursery care unit in AL-Kadimiya Teaching Hospital, identification of bacterial agents causing early and late onset neonatal sepsis and their antimicrobial susceptibility to commonly used antibiotics in this hospital, and the short term outcome from neonatal septicemia and its relation to the gender, birthweight, gestational age, and onset of sepsis.

Patients and methods

This prospective study was performed on 664 neonates with clinical signs and symptoms suggesting sepsis (such as feeding intolerance, apnea ,lethargy , cyanotic spells , respiratory distress , or suggestive perinatal history of infection). Number of confirmed cases of neonatal sepsis by positive blood cultures was 105 neonates . Patients were collected from neonatal care unit of AL-Kadimiya Teaching hospital from the 1st of January to the end of October 2011.

All neonates admitted to neonatal care unit with signs and symptoms suggestive of septicemia that were confirmed by a positive blood culture

(excluding those who received antibiotics) were enrolled in this study (total number=105 neonates). Patients were classified on the basis of onset of symptoms in relation to the age into: Early onset neonatal sepsis (EONS) i.e. from birth up to 3 days, and late onset neonatal sepsis (LONS) i.e. more than 3 days and up to 28 days.

Data were collected include: Gestational age, Birth weight , Gender, Onset of sepsis, Place of delivery and also we followed up the subjects and recorded the outcome till discharge.

Povidone iodine solution was applied to the skin over the area selected for blood aspiration, saturated cotton starting centrally on the planned site exerting moderate and moving out in concentric circles. The iodine was allowed to dry then was removed with sponges saturated with 70% of propyl alcohol; blood from each neonate was withdrawn from peripheral vein before antibiotic therapy. A specimen of 2 ml of blood was taken in a small culture media bottle containing 25 ml of brain heart liquid broth, vitamin k1 and anticoagulant (the media for growth of bacteria) , then incubated at 37c for at least 72 hours.

After 3 days, the sample was taken from the media which contained 2 ml of patient's blood streaked in Blood Agar, Chocolate Agar and Macconkey Agar for 24 hours in 37C and watched to see if microorganisms grow.

Note: In this study we identified aerobic bacteria only because we do not have the materials to identified anaerobic bacteria. The identification of causative micro-organisms were based on colonial morphology and any change exhibited on the media like hemolysis, pigmentative lactose fermentation or non lactose fermentation then staining reaction using Grams stain which classify micro-organism into Gram-positive and Gram-negative. All the procedures were performed by expert bacteriologist.

If there was growth we did subculture to identify specific micro-organism and doing sensitivity tests to know the sensitive and resistant antibiotics.

Each patient suspected of having septicemia received a combination of ampicillin (100mg/kg) or ampicillin/cloxacillin (200 mg /kg) and gentamycin (5 mg /kg). Another combination include ampicillin or ampicillin/cloxacillin and cefotaxime (100mg/kg) also used as an empirical treatment in this hospital. This therapy was later modified according to culture and susceptibility results.

Statistical analysis was performed using SPSS version 10. Number and percentages were used for categorical variables, The Pearson Chi Square test was used for categorical variables to measure outcome differences between

sepsis survivors and non-survivors. A P value less than (0.05) was considered significant.

Results

In this prospective study and from 664 neonate with sign and symptoms of neonatal sepsis were admitted, positive blood cultures were obtained for 105 neonates (15.8%). Among105, 63(60%) had sepsis with gram negative bacteria and 42 (40%) with gram positive bacteria. Gram negative bacteria were predominant in both early (66.7%) and late (56.9%) onset neonatal sepsis. From 105 cases, the most common isolated gram negative bacteria were E.coli (24.8%), Enterobacter spp.(16.2%), Pseudomonas (8.6%) and Klebseilla (7.6%), while the most common isolated gram positive bacteria were CONS (18.1%), Staphylococcus epidermidis (12.4%) and Staphylococcus aureus (9.5%). Other microorganisms (total number=3), like Proteus and Staphylococcus albus showed no results of their susceptibility to the used antibiotics were neglected in this study. Among neonates with sepsis, 33 patients (31.4%) had early onset and 72 patient (68.6%) had late-onset neonatal sepsis (table 1) and (figure 1).

Based on the results from susceptibility testing, the sensitivity of the isolated causative agent to selected antibiotics were the same in early and late onset sepsis in this study, ***E.coli*** was 73% resistant to ampicillin and 100% resistant to cloxacillin but show 65.4% sensitivity to gentamicin and 57.7% sensitivity to cefotaxime. ***Enterobacter spp***. show 100% resistant to ampicillin and cloxacillin, 64.7% resistant to cefotaxime but show 76.5% sensitivity to gentamicin. ***Pseudomonus a.*** show 100% resistant to ampicillin and cloxacillin, 66.7% resistant to gentamicin but show 66.7% sensitivity to cefotaxime. ***Klebsiella pneumonia*** show 100% resistant to ampicillin and cloxacillin , 87.5% resistant to gentamicin and 75% resistant to cefotaxime . All of the isolated ***gram positive bacteria*** were mostly sensitive to ampicillin, cloxacillin and cefotaxime but highly resistant to gentamicin as in table (2), figure (2) and figure (3).

Among 105 newborns with sepsis, 62(59.1%) were preterm and 43(40.9%) were term, there were 56 (53.3%) neonates with low birth weight and 49 newborns (46.7%) with normal birth weight, there were more cases of sepsis in male neonates compared with female neonates [63(60%) male and 42(40%) female]. Regarding place of delivery, 74 (70.5%) cases were delivered at hospital and 31 (29.5%) at home (table 3).

The death rate was 20.9% (22 neonates died: 13 with early onset sepsis and 9 with late onset sepsis,13 male and 9 female, 21 with gestational age less than 37 weeks and 1 with gestational age ≥ 37 weeks, 19 with birth weight less than 2500 gm and 3 with birth weight ≥ 2500 gm). Early onset sepsis, male gender and gestational age less than 37 weeks were found to be significantly

associated with death (P value=0.002 of each one), also birth weight less than 2500 gm was found to be significantly associated with death (P value=0.001) (table 4).

Table(1). Type and number of bacterial isolates in neonates with sepsis based on the sepsis onset.

Microorganisms	Early-onset(%)	Late-onset(%)	Total(%)
Gram-negative bacilli			
Enterobacter	6 (18.2)	11 (15.2)	17 (16.2)
Klebseilla	4 (12.1)	4 (5.6)	8 (7.6)
E.colli	9 (27.3)	17 (23.6)	26 (24.8)
Pseudomonus	0 (0)	9 (12.5)	9 (8.6)
Others	3 (9.1)	0 (0)	3 (2.8)
Total No. of gram negative bacteria	***22 (66.7)***	***41 (56.9)***	***63 (60%)***
Gam-positive cocci			
CONS	4 (12.1)	15 (20.8)	19 (18.1)
Staphylococcus epidermidis	4 (12.1)	9 (12.5)	13 (12.4)
Staphylococcus aureus	3 (9.1)	7 (9.8)	10 (9.5)
Total No. of gram positive bacteria	***11 (33.3)***	***31 (43.1)***	***42 (40%)***
Total No.(%)	**33 (31.4)**	**72 (68.6)**	**105 (100)**

Note : Others= **Proteus**(No.=1), **staphylococcus albus**(NO.=2) had no results of their suseptability to the used antibiotics.

No. of Patients

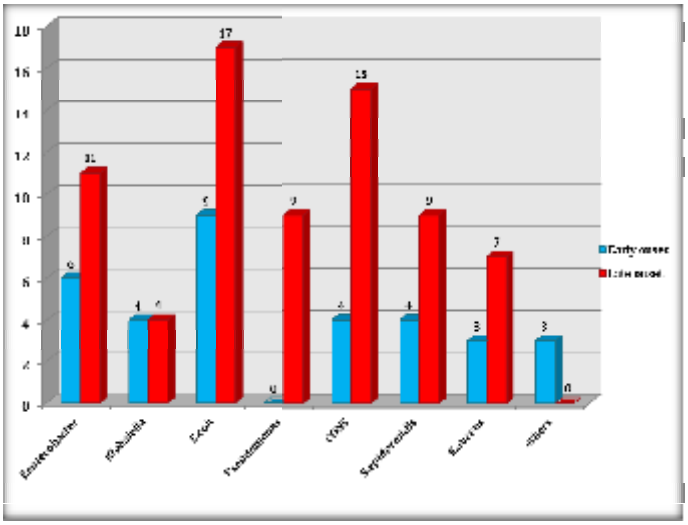

Figure (1). Type and number of bacterial isolates in neonates with sepsis based on the sepsis onset .

Table(2). Antimicrobial susceptibility pattern of bacteria to selected antibiotics.

Microorganisms (Total No.)	Antibiotics			
	Ampicillin	**Cloxacillin**	**Gentamicin**	**Cefotaxime**
E.coli (26)	7 (27%)S 19 (73%)R	0(0%)S 26(100%)R	17(65.4%)S 9(34.6%) R	15(57.7%)S 11(42.3%)R
Enterobacter (17)	0 (0%) S 17(100%)R	0(0%)S 17(100%)R	13(76.5%)S 4(23.5%)R	6(35.3%)S 11(64.7%)R
Pseudomonas (9)	0(0%) S 9(100%)R	0(0%)S 9(100%)	3(33.3%)S 6(66.7%)R	6(66.7%)S 3(33.3%)R
Klebsiella (8)	0(0%) S 8(100%)R	0(0%)S 8(100%)R	1(12.5%)S 7(87.5%)R	2(25%)S 6(75%)R
CONS (19)	19(100%)S 0(0%)R	15(79%)S 4(21%)R	6(31.6%)S 13(68.4%)R	16(84.2%)S 3(15.8%)R
Staphylococcus epidermidis (13)	8(61.5%)S 5(38.5%)R	7(53.8%)S 6(46.2%)R	6(46.2%)S 7(53.8%) R	10(77%)S 3(23%)R
Staphylococcus aureus (10)	7(70%)S 3(30%)R	10(100%)S 0(0%)R	0(0%)S 10(100%)R	8(80%)S 2(20%)R

Total No.=Total Number, S=Sensitive, R=Resistant.

Percent(%)

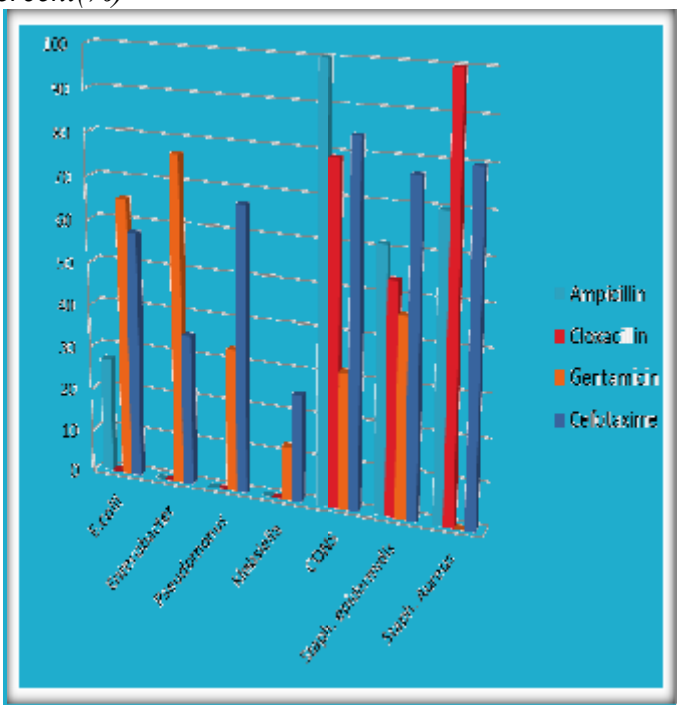

Figure (2). Antimicrobial sensitivity pattern of bacteria to selected antibiotics.

Percent(%)

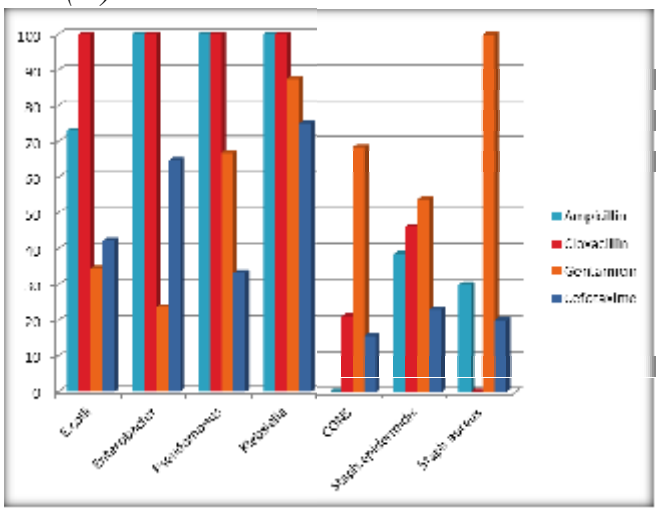

Figure (3). Antimicrobial resistant pattern of bacteria to selected antibiotics.

Table (3).Characteristics of total number of neonates infected with microorganisms.

Variable	Frequency	%
Sex		
Male	63	60
Female	42	40
Gestational age		
< 37 weeks	62	59.1
≥ 37 weeks	43	40.9
Birth weight		
< 2500 gm	56	53.3
≥ 2500 gm	49	46.7
Onset of sepsis		
Early onset sepsis	33	31.4
Late onset sepsis	72	68.6
Place of delivery		
Hospital	74	70.5
Home	31	29.5
Outcome		
Died	22	20.9
Survived	83	79.1

Table (4).Outcome and risk factors associated with death

Variable (Total No.)	Survivors(N=83)	Nonsurvivors(N=22)	P value
	No.(%)	**No.(%)**	
Birth weight<2500 gm (56)	37 (44.6)	19 (86.4)	0.001
Birth weight ≥2500 gm(49)	46 (55.4)	3 (13.6)	0.972
Gestational age <37 weeks (62)	41 (49.4)	21 (95.5)	0.002
Gestational age ≥ 37 weeks (43)	42 (50.6)	1 (4.5)	0.989
Male (63)	50 (60.2)	13 (59.1)	0.002
Female (42)	33 (39.7)	9 (40.9)	0.922
Early onset sepsis (33)	20 (24.1)	13 (59.1)	0.002
Late onset sepsis (72)	63 (75.9)	9 (40.9)	0.922

N= number, Total No.=Total Number.

Discussion

In this study, the percent of documented neonatal sepsis with positive blood culture was 15.8%. This percent was much lower than the percent of positive blood cultures in Rahman et al. (2002) study (62.8%) (8), and Bhttacharjee et al. (2008) study (48%) (9). The lower prevalence of documented neonatal sepsis with positive blood culture in this study had different reasons such as antibiotic administration in mother, difficulty in sampling, blood culture technique (Bansal et al., 2004) (10), or sepsis due to

anaerobic, viral or fungal pathogens(Agnihotri et al., 2004) (11), and misdiagnosis because of some similarities between the clinical signs of sepsis with other diseases like metabolic disorders (Lund et al., 2002) (12). Other studies show similar incidence at other teaching hospitals in Baghdad like Ibrahim AH. (2005) study (15.5%) (13), and Al-Shawi BA. (2006) study (9.3%) (14). Other workers reported much higher rates in other developing countries like Das Pk. Et al. (1999) study (36%) (15), and Ako-Nai Ak. et al. (1999) study (55%) (16), and lower rates (1-5 per 1000 live births) in developed countries (Escobar GJ. et al., 2002) (17). In current study, late onset sepsis was more common than early onset sepsis (68.6% versus 31.4%). Such figure is comparable to that reported by Bilal N. at Saudi Arabia (63.8%) (18), higher than that from USA (51%) by Sarasohn C.(19), and lower than that reported by Siegel J. in London (70.9%) (20). This finding was also similar to the results of the Kuruvilla et al. (1998), who reported the higher prevalence of late-onset sepsis compared with early-onset (77.1 versus 22.9%) (21). On the contrary,Vinodkumar et al. (2008) study reported higher prevalence of early onset neonatal sepsis (73%) (22).

In this study and in both early and late onset neonatal sepsis, gram negative bacteria was the most common (60%) causative agent [*E.colli* show greater number (24.8%) then *Enterobacter* (16.2%)]. Coagulase-negative Staphylococcus were the next most common gram positive bacteria followed by Staphylococcus epidermidis and staphylococcus aureus. These results were in agreement with other studies in Australia (Isaacs D., 1999) (23), Sundaram et al (2009) in India (24), also in Emirates (Koutouby A., 2007) (25). This finding was dissimilar to the results of a recent study from Iran showing the CONS as the most common isolated bacteria (Gheibi et al., 2008) (26). As the majority of cases in this study were of late onset sepsis and were delivered in a hospital , nosocomial infection is possible. Sources of infection might include mothers , nursing staff or equipment .

No GBS colonies were isolated from cultures in this study as previously reported from other hospital in Baghdad and developing countries (13,14,27). This may be due to lower colonization of pregnant mothers with GBS or weak virulence of these bacteria(27). In contrast, the incidence of group B streptococci is 3.6 per 1000 live births in UK (Anthony S. Fauci, 2008) (28), and other developed countries, which have a high rate of vaginal colonization with group B streptococci like in Isaacs et al (1995) study (29), Dutta S. et al (2010) study (30), and Baffour GF. et al (2009) study(31) .Differences in vaginal colonization rates between woman in developed and developing countries may be the reasons for this variation.

A large number of gram negative and gram positive bacteria, were resistant to one or more type of antibiotics which was in agreement to similar studies like Lund et al. (2002) (12), Vinodkumar et al. (2008) (22), and Issacs et al (2006)

(32) . Nowadays antibiotic resistance is a widespread global problem that caused ineffectiveness of current empirical treatment against gram negative bacteria. Antibiotic resistance can cause many difficulties in the treatment of sepsis such as increase in death rate, duration of hospitalization and treatment expenses. So it is necessary that antibiotic treatment program is reevaluated continuously (33). The study showed that males were affected more than female neonate in a percentage of (60 % versus 40 %), those with gestational age less than 37 weeks were affected more than those with gestational age more than or equel to 37 weeks (59.1% versus 40.9%), those with birth weight less than 2500 gm were affected more than those with birth weight more than or equel to 2500 gm (53.3% versus 46.7%) and those delivered at hospital were more than those delivered at home (70.5% versus 29.5%), these figures are comparable to data reported by other workers, like Obi JO Kafrawi MM (1999) (34), and Buetow Kc (2002) (35), also close to the results of Mosayebi et al. (2003) study (36). such results suggest the possibility of sex linked factor in host susceptibility, immaturity of immune system in those with gestational age less than 37 weeks and in those with birth weight less than 2500 gm, and more susceptibility to nosocomial infection in those delivered at hospital (37). The death rate in this study was (20.9%) which was lower compared with that reported in Khassawneh et al. study (2009) which was reported as 30.9% (38), but higher than that reported by Adams-Chapman I, et al (2002),who report 10% mortality rate of neonatal sepsis (39), and also higher than that reported in Taiwan (14%) by Ni-Chung Lee et al. (2004) (40).The high death rate in this unit probably reflect suboptimal perinatal care, late presentation and unhygienic umbilical cord (40).

Conclusions

The percent of documented neonatal sepsis with positive blood culture was 15.8%. Gram negative bacteria was the main cause of early and late-onset neonatal sepsis in this NCU and *E.colli* was the most common pathogens, while the most common isolated gram positive bacteria was CONS. Many of the isolated bacteria from sepsis were resistant to the used antibiotics. Low birth weight neonates <2500 gm, gestational age < 37weeks, male gender and early onset sepsis were significantly associated with death. The death rate due to neonatal sepsis was higher compared with the other studies.

Recommendations

Continuous surveillance of neonatal sepsis in order to follow closely changes in trends and risk factors, to obtain information for empiric antibiotic therapy and to react rapidly in case of major changes in susceptibility patterns and occurrence of outbreaks. Minimizing invasive procedures as much as possible and ideal nursery setup which includes adequate space for care of

infants and aseptic equipments for monitoring. The possible changing nature of the bacteria pathogens at the unit needs further monitoring and the results of this study needs periodic reviewing, together with determining the antibiotic sensitivity pattern. The poor out come of neonate with septicemia make it mandatory to constantly review the pattern of pathogen.

References

1. Behrman R.,Klinkman R., and Jenson H. , saunders company . "Infections of neonatal infants" . Nelson Textbook of pediatrics.19th ed.2011:98;623 .
2. Bang AT, Bang RA. Et al. Effect of home based neonatal care and management of sepsis on neonatal mortality: field trial in rural India. Lancet 1999; 354:1955-61.
3. Stoll BJ. The global impact of neonatal infection. Clin Perinatol 1997;24:1-21.
4. Bang AJ, Bang RA, Baitale B. Burden of morbidities and unmet need for healthcare in rural Indian neonate. Pediatr 2001; 38:952-65.
5. Klein JO, Remington JS; current concepts of infections of the fetus and newborn infant. In Remington JS, Klein JO (editors;infectious deseases of the fetus and newborn infants) 5th ed philadelphia, WB Saunders, 2001;p1 – 32.
6. Singh M. Perinatal Infections. In Singh M, ed. Care of the Newborn. 5th Ed. New Delhi: Interprint; 1999; 198-221.
7. Kliegman RM, Marcdanate KJ, Behrman RE, et al. Elsevier Saunders. "Sepsis and Meningitis Nelson Essentials of Pediatrics" Fifth ed. 2006: 326-329.
8. Rahman S, Hameed A, Roghani MT, Ullah Z. Multidrug resistant neonatal sepsis in Peshhawar, Pakistan. Arch. Dis.Child. Fetal Neonatal Ed. 2002; 87(1): F52-F54.
9. Bhttacharjee A, Sen MR, Prakash P, Gaur A, Anuprba S.Increased prevalence of extended spectrum B Iactamase producers in neonatal septicaemic cases at a tertiary referral hospital. Indian J. Med. Microbiol. 2008; 264(4): 356-360.
10. Bansal S, Jain A, Agarwal J, Malik GK. Significance of coagulase negative staphylococci in neonates with late onset septicemia. Indian J. Pathol. Microbiol. 2004; 47(4): 586-568.
11. Agnihotri N, Kaistha N, Gupta V. Antimicrobial susceptibility of isolates from neonatal septicemia. JPN. J. Infect.Dis.2004;57(6):273-275.
12. Lund AM, Christensen E, Skovby F. Diagnosis and acute treatment of inborn metabolic diseases in infants. Ugeskrift for Laeger. 2002;164(48): 5613-5619.

13. Ibrahim AH. Bacterial septiciemia in neonate . J fac Med Bagh 2005; 47:162-64.
14. Al-Shawi BA , Al-Hadith TS , Al-Abasi, etal. Neonatal infection in the neonatal unit at Baghdad Teaching Hospital , Iraq. IPMJ 2006;5:295-97.
15. Das Pk , Basak , Chakraborty P, etal. Clinical and Bacteriological profile of neonatal infection in Metroprofilan City based medical college nursery . J Ind Med Assoc 1999;97:35.
16. Ako-Nai Ak , Adejuighi EA, Ajayi FM, etal. The bacteriology of neonatal septicemia in Ueffe, Nigeria . J trop Paed 1999;45:146-51.
17. Escobar GJ, Dekun Li, Armstrong MA , etal . Neonatal sepsis workups in infants > 2000 grams at birth . Pediatrics 2000;106:256-65.
18. Asindi A.A.,Bilal N. E.,et al."Neonatal septicemia".Saudi medical Journal.2004;25(14):822-25.
19. Sarasohn C. "Care of very small premature infants". Pediatrics clinic of north America 2000;28(3):631-33.
20. Siegel J. and McCracken G."Sepsis neonatorum" . New England Journal of pediatrics 2005;90(19):159-63 .
21. Kuruvilla KA, Pillai S, Jesudason M, Jana AK (1998).
Bacteriological profile of sepsis in a neonatal unit in south India. Indian Pediatr., 35:851-858.
22. Vinodkumar CS, Neelagund YF, Suneeta K, Sudha B,KalapannavarNK, Basavarajapa KG (). Perinatal risk factors and microbialprofile of neonatal septicemia: A multicentred study. J. Obstet.Gynecol. India 2008; 58(1): 32-40.
23. Isaacs D, Royal JA . Intrapartum antibiotics and early-onset neonatal sepsis caused by group B streptococcus and by other organisms in Australia. Australasian Study Group for Neonatal infections. Pediatr. Infect. Dis. J. 1999; 18: 524-528.
24. Sundaram V, Kumar P, Dutta S, Mukhopadhyay K, Ray P, Vikas Gautam V, Narang A. Blood Culture Confirmed Bacterial Sepsis in Neonates in a North Indian Tertiary Care Center: Changes over the Last Decade Jpn. J. Infect. Dis. 2009; 62(1): 46-50.
25. Koutouby A., and Habibulla J. " Neonatal sepsis in Dubai ,United Arab Emirates" . Journal of tropical pediatrics 2007;21(28):251-53 .
26. Gheibi S, Fakoor Z, Karamyyar M, Khashabi J, Ilkhanizadeh B, Asghari-Sana F, Mahmoodzadeh H, Majlesi AH. Coagulase Negative Staphylococcus; the Most Common Cause of Neonatal Septicemia in Urmia, Iran. Iranian J. Pediatr. 2008; 18(3): 237-243.
27.Payman S, Ali-Akbar R, Massod Y, etal. Neonatal nosocomial infection in Bahrami Children Hospital. Indian J pediatr 2006;78:197-200.

28. Anthony S. Fauci, Dennis L. Kasper, Dan L. Longo, et al. Severe sepsis and Septic Shock. Harrison's Internal Medicine.17th ed.2008; 265.
29. Isaacs , Barfield C ,Grimwood K, etal. Systemic bacterial and fungal infections in infants in Australian neonatal unit . Med J Aust 1995;162:198-200 .
30. Dutta S, Reddy R, SheilkhS, etal. Intrapartum antibiotics and risk factors for early onset sepsis . Arch Child Fetal Neonatal Ed 2010;95:F99-F103.
31. Health PT, Baffour GF, Tighe H, etal .Group B streptococcal disease in infants :a case control study. Arch Dis Child 2009;94:674- 80.
32. Isaacs D.Unnatural selection :reducing antibiotic resistance in neonatal units.Arch Dis Child Fetal NeonatalEd 2006;9:F72-4.
33. Goossen H. Antibiotic resistance and policy in Belgium. Verh. K. Acad. Geneeskd. Belg. 2000; 62: 439-469.
34. Obi JO Kafrawi MM, Ignacio LC. "Neonatal septicemia". Saudi med. J 1999; 20(6):433-437.
35. Buetow Kc, Klein Sw, Lane RB. Septicemia in premature infant Amer. J. Dis Child.2002.110:29-41.
36. Mosayebi Z, Movahedian AH, Moniri R. Profile of Bactrial Sepsis in Neonates from Kashan in Iran. J. Infect. Dis. Antimicrob. Agents. 2003;20: 97-102.
37. Behrman R.,Klinkman R., and Jenson H., Nelson textbook of pediatrics" . 18th ed. 2007: 623-640 and 846-850.
38. Khassawneh M, Khader Y, Abuqtaish N. Clinical features of neonatal sepsis caused by resistant Gram-negative bacteria.Pediatr.Inter.2009; 51(3): 332-336.
39. Adams-Chapman I, Stoll BJ: prevention of nosocomial infection in the neonatal intensive care unit, Curr Open pediatr 2002; 14: 157.
40. Ni-Chung Lee, Shu-Jen Chen, Ren-Bin Tang, Be-Tau Hwang;Veterans General Hospital, Taipei. Department Of Pediatrics, National Yang-Ming university, Taiwan, R.O.C..original article,neonatal bacteremia in neonatal intensive care unit. J.China Medical Association 2004;67: p.208-15.