Retrospective Analysis of Neonatal Bacteremia and Antimicrobial Resistance Pattern in Neonatal Intensive Care Unit

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ABSTRACT

Objective: To study the distribution of bacterial pathogens and their antibiotic resistance pattern in neonatal intensive care unit and the preventive measures to prevent spreading of multidrug resistant strains.

Setting: The study was conducted at Neonatal Intensive Care Unit of Cairo University Hospitals during the period from May 2010 to June 2011.

Methods: 1078 Blood specimens for culture were drawn from 589 newborns admitted in a NICU with suspected sepsis. The specimens were inoculated into brain heart infusion broth. Subcultures were performed on standard media and the isolates were identified by standard biochemical tests. Antibiotic susceptibility pattern of the isolates was studied by Modified Kirby Bauer disc diffusion technique. Percentage of antibiotic resistance was calculated.

Results: Blood culture positive was found in 74 cases from 589 (13%). They included Acinetobacter spp. (14), Klebsiella spp. (15), mixed infection with the 2 previous organisms (1), Coagulase negative Staphylococci CONS (34), Methicillin resistant Staphylococcus aureus MRSA (4), Streptococcus viridans (5), Enterococcus spp. (1). 75% of Acinetobacter species were pan-drug resistant PDR to all available antibiotics, Klebsiella spp, were multidrug resistant MDR and sensitive to carbapenems (73%) and amikacin (67%). CONS, MRSA, streptococcus viridans and enterococcus spp were all sensitive to vancomycin. The mortality was seen in 11 cases (14.9%), and was mainly associated with gram negative organisms (64%).

Conclusion: The resurgence of multi-drug resistant strains is an important issue especially in NICU. To control it we need to optimize the use of antimicrobial therapy, to implement antibiotic policy programs and guide antimicrobial choice in cases when prophylactic or therapeutic therapy must be started before blood culture and antibiogram results are available, due to the clinical conditions of the patients, we need also to control the infections and limit spreading of MDR or PDR organisms by the emphasize and stress on the practices of prevention control measures.

Key words: Early and late onset, bacteremia, neonate, antimicrobial resistance.

Introduction

Neonatal infections are important cause of morbidity, prolonged hospital stay and mortality among infants, particularly those born preterm and of very low birthweight VLBW(Adams-Chapman and Stoll, 2006). Factors associated with neonatal sepsis include foetal distress; low Apgar score, requirement of mechanical ventilation; umbilical catheterisation and history of pre eclampsia in mothers (Dawodu, A. et al., 1997, Mahmood, A. et al., 2002).

Pathogens causing neonatal infections and their antibiotic susceptibility patterns may change over time (May, et al., 2005) and differ between countries (Zaidi et al., 2005). It is extremely important to diagnose the cases in time so that appropriate antibiotic treatment can be given. Moreover, the bacterial pathogens responsible and their susceptibility pattern should be regularly monitored in a hospital setting (Mahmood, A. et al., 2002). It is therefore essential to monitor the epidemiology of neonatal infections to inform policy and clinical practice. Considering the high infection rate among patients, their high treatment expenses, the high rate of mortality and the resistance of these hospital pathogen microorganisms to antibiotics, specific attention and proper measurement especially in the prevention of nosocomial infections is quite necessary (Mahbobe, et al., 2007). Neonatal infection surveillance networks have been established in several countries and are useful for documenting changes in clinical practice, monitoring changes in pathogens and their antibiotic resistance over time, informing policy and improving quality of care (Gray, 2007).

Aim of the Research:

The aim of the study is to describe the distribution of the different bacterial agents as a cause of bacteremia in neonatal intensive care unit NICU, and their antibiotic susceptibility pattern, consequently guidelines for

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empiric antibiotic policy can be prepared, and effective implementation of infection control measures could be
tailored to prevent spreading of multi-drug resistant bacterial strains in the NICU.

Data Collection:

This retrospective study was carried out from May 2010 to June 2011 by reviewing blood cultures results of
neonates in neonatal intensive care unit (NICU) of Gynaecology and Obstetric Hospital, Cairo University
Hospitals. The total no of cases admitted during this period was 589.

Data from patients with positive blood cultures were collected from neonatal case records, and included
date of admission, age, sex, clinical diagnosis on admission, date of first positive blood culture, mortality, the
pathogenic organism isolated and their antibiotic susceptibility pattern.

Materials and Methods

1078 Blood culture samples were sent for 589 cases (2 samples from each patient one on admission the
second after five days from admission) with risk factors or suspected of having systemic infection. “Early onset”
sepsis, if it presents during the first 5-7 days of life and “Late onset” sepsis if it occurs after the first week
(Mahmood, A et al., 2002), we considered early onset sepsis if the first sample of blood culture is positive and
late onset if the second sample is positive and the first is negative. The bottles used for blood cultures held 40 ml
of brain heart infusion broth (BD Bactec Peds Plus/F). 1-3 ml of blood was collected by using aseptic
technique and added to the broth, then the bottles were labeled and sent to the main microbiology laboratory,
they were incubated at 35°C in BACTEC 9240 (Becton Dickinson Microbiology Systems, Sparks, Md, US) for
5 days. Bacterial growth was detected by inducing alarm, then cultures on standard media were done on Blood,
Cholate and MacConkey to isolate the micro-organism, incubate the first two plates in 10% CO2 and the third
aerobically. Identification was done by Gram stain, biochemical reactions, and antimicrobial sensitivity testing
was done on Mueller Hinton agar by Kirby Bauer disc diffusion technique according to the recommendation of
Clinical Laboratory Standard Institute (CLSI, 2006). Gram positive and Gram negative antibiotic discs were
used according to the organism isolated, the results were reported as sensitive (S), Intermediate (I) or Resistant
(R).

Gram positive discs incuded Ampicillin, Oxacillin, Amoxicillin-Clavulanic acid, Clindamycin,
Deoxychylcine, Vancomycin, Erythromycin, Ciprofloxacin, aminoglycosides, rifampin, lincomycin. Gram
dnegative discs included Ampicillin, Gentamicin, Amikacin, Meropenem, Cefuroxime, Cefotaxime, Cefazidime,
Ciprofloxacin,Amoxicillin-Clavulanic acid, Co trimoxazole, Pipracillin- Tazobactam, Cefoperazone-
Sulbactam.

Results and Discussions

Positive blood cultures (first result) were found in 74 from 589 neonates (13%), they were less than 35 days
of age (average 7.4) 37 male and 37 female, 67(90.5%) were diagnosed as respiratory distress syndrome and 7
(9.5%) were diagnosed as transient tachypnea of newborns (TTN), aspiration meconium and neonatal jaundice.
The EONS (Early Onset Neonatal Sepsis) accounted for 33 (44.6%) and LONS (Late Onset Neonatal Sepsis)
accounted for 41 (55.4%). The most prevalent organism isolated from blood cultures was CONS (46%) in both
early and late onset infections, whereas Enterococcus spp. was the least prevalent and was isolated from only
one case in late onset infection (2.4%) (Table 1). Organisms isolated during late onset sepsis comprised about
half of total isolates. Gram positive organisms were seen mainly in early onset however gram negative
organisms were seen mainly in late onset infection. The type of bacteria isolated from early and late onset
infections were summarized in Table 1.

Table 1: Total no of isolates from Blood Culture, early and late onset.

<table>
<thead>
<tr>
<th>Organism</th>
<th>N= 74 (%)</th>
<th>Early Onset N= 33 (44.6%)</th>
<th>Late onset N= 41 (55.4%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter spp.</td>
<td>14(19)</td>
<td>5(15)</td>
<td>9(22)</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>15(20)</td>
<td>4(12)</td>
<td>11(27)</td>
</tr>
<tr>
<td>Acinetobacter + Klebsiella</td>
<td>1(1)</td>
<td>1(3)</td>
<td>0</td>
</tr>
<tr>
<td>CONS</td>
<td>34(46)</td>
<td>18(55)</td>
<td>16(39)</td>
</tr>
<tr>
<td>MRSA</td>
<td>4(5)</td>
<td>3(9)</td>
<td>1(2.4)</td>
</tr>
<tr>
<td>Strept. Viridans</td>
<td>5(7)</td>
<td>2(6)</td>
<td>3(7.3)</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>1(1)</td>
<td>0</td>
<td>1(2.4)</td>
</tr>
</tbody>
</table>

No B hemolytic streptococci was isolated during the study period.

Out of 74 positive blood cultures, 30 cases showed gram negative organisms (40 %) and 44 cases showed
gram positive organisms in their blood cultures(60%), details of percentage of organisms isolated in both early
& late onset sepsis are presented in Table 1.
Gram positive organisms included CONS, MRSA, Strept. viridans and enterococci, they were sensitive to vancomycin, few isolates of CONS were sensitive to oxacillin. Antibiogram of Gram positive organisms isolated from blood cultures was mentioned in table 2.

Acinetobacter pan drug resistant PDR (resistant to all available antibiotics) resistant to ampicillin, cephalosporins, amoxicillin-clavulanic acid, co trimoxazole, carbapenems, fluoroquinolones, gentamicin, was isolated from 11 cases, few cases were sensitive to amikacin (3), cefoperazone-sulbactam (4) and piperacillin-tazobactam (3). Klebsiella MDR was isolated from 15 cases, resistant to ampicillin, cephalosporins, cotrimoxazole, cefoperazone-sulbactam, piperacillin-tazobactam, amoxicillin-clavulanic acid, quinolones, sensitive to amikacin and carbapenems in 10 cases only. Antibiograms of Gram negative bacteria was mentioned in Table 3.

### Table 2: Antibiotic resistance pattern of Gram positive bacteria.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>CONS (n=34)</th>
<th>Streptviridans (n=5)</th>
<th>MRSA (n=4)</th>
<th>Enterococcus spp (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>R/S 34/0</td>
<td>% R 100</td>
<td>R/S 4/0</td>
<td>% R 100</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>30/4</td>
<td>88</td>
<td>4/1</td>
<td>80</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>6/28</td>
<td>18</td>
<td>---</td>
<td>1/3</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>28/6</td>
<td>82</td>
<td>3/2</td>
<td>60</td>
</tr>
<tr>
<td>Amikacin</td>
<td>9/25</td>
<td>26</td>
<td>4/1</td>
<td>80</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>19/15</td>
<td>56</td>
<td>---</td>
<td>1/3</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>26/8</td>
<td>76</td>
<td>---</td>
<td>3/1</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0/34</td>
<td>0</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>29/5</td>
<td>85</td>
<td>4/1</td>
<td>80</td>
</tr>
<tr>
<td>Amoxicillin/Clavulinc a</td>
<td>30/4</td>
<td>88</td>
<td>2/3</td>
<td>40</td>
</tr>
<tr>
<td>Rifampin</td>
<td>3/31</td>
<td>9</td>
<td>---</td>
<td>0/4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>18/16</td>
<td>53</td>
<td>3/2</td>
<td>60</td>
</tr>
</tbody>
</table>

### Table 3: Antibiotic resistance pattern of isolated Gram negative bacteria.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Acinetobacter spp (n=15)</th>
<th>Klebsiella spp. (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>R/S 15/0</td>
<td>% R 100</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>13/2</td>
<td>87</td>
</tr>
<tr>
<td>Amoxicillin/Clavulinc a</td>
<td>15/0</td>
<td>80</td>
</tr>
<tr>
<td>Amikacin</td>
<td>12/3</td>
<td>80</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>15/0</td>
<td>100</td>
</tr>
<tr>
<td>Cefazidine</td>
<td>13/2</td>
<td>87</td>
</tr>
<tr>
<td>Ceftazidine</td>
<td>14/1</td>
<td>93</td>
</tr>
<tr>
<td>Meropenem</td>
<td>13/2</td>
<td>87</td>
</tr>
<tr>
<td>Imipenem</td>
<td>13/2</td>
<td>87</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>15/0</td>
<td>100</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>14/1</td>
<td>93</td>
</tr>
<tr>
<td>Piperacillin/taizobactam</td>
<td>12/3</td>
<td>80</td>
</tr>
<tr>
<td>Cefoperazone/sulbactam</td>
<td>11/4</td>
<td>73</td>
</tr>
</tbody>
</table>

S= Susceptible  R= Resistant.

The overall mortality was seen in 11 cases (14.9%), and was mainly associated with Acinetobacter and klebsiella species (64 %), CONS (18 %), MRSA (9 %), Strept. viridans (9 %). The mortality in early onset sepsis was 5/33 (15.2 %) and was similar to that in late onset sepsis 6/41 (14.6 %) (p=0.950). The mortality was insignificant in male babies by comparison to female babies with sepsis (p value 0.327). The mortality due to Gram negative sepsis (64%) was significantly more than Gram positive sepsis (36%).

**Discussion:**

Neonatal sepsis is a life-threatening emergency and any delay in the treatment may be fatal(Yurdakok, M., 1998). Appropriate treatment with antibiotics requires knowledge of common bacterial pathogens involved and their antibiotic sensitivity pattern. The overall sepsis rate in the study period was 13% (74/589 cases), some researchers gave a figure of 7.4% of neonatal intensive care unit admissions (Khadilkar, et al., 1995 and Hafsa et al., 2011).

The lons (Late Onset Neonatal Sepsis) was more common than EONS (Early Onset Neonatal Sepsis) which is compatible with a previous report from Bangladesh reporting that LONS (Late Onset Neonatal Sepsis) was more common within the rural population of Bangladesh without absence of specialized neonatal care facilities (Ahmed et al, 2002). But this was in contrast with reports from the other developing countries that reported that EONS was more common than LONS (Fisher et al., 1983; Vesicari et al., 1985).
The Majority of isolates found in early onset sepsis in our study included Coagulase negative staphylococci (CONS) responsible for half of the cases (54%) and included contamination or suspected infection, gram negative rods (Acinetobacter and Klebsiella spp.) was responsible for about third of the cases (30%), and other gram positive organisms including Staph MRSA, Strept.viridans and enterococci account for 16% of cases. Group B Streptococci was not isolated from any of the cases. Our findings are similar to several studies that shown that gram positive bacteria are the most common organisms causing septicemia in NICU and CONS had the highest rate (Burnie et al., 1997, Miragaipe et al., 2002, Krediet et al., 2004, Mahbobeh et al., 2007). In contrast to the findings of Mahmood et al. (2002), where the majority of isolates causing early onset sepsis were gram negative rods. The newborns most probably acquire the gram-negative rods from the vaginal and faecal flora of the mother and the environment where the delivery occurs. Importance of both vertical transmission from the mother and postnatal acquisition of infection from the environment has been suggested in the pathogenesis of neonatal sepsis by Bhutta and Yusuf in 1997 and Palazzi et al. 2006. In our study early onset sepsis may be acquired from maternal emergency department : maternal fecal flora during delivery, servo area or the place where there is immediate care of baby after delivery, crowding of babies (more than one on a servo) with low number of nurses, instruments used, attendants, lack of adherence to infection control measures due to high number of patients.

Schabery et al., 1991 have found that gram positive bacteria were recognized as the most important cause in nosocomial infections and mortality. Similarly in our study we found that the highest rate of organisms isolated both in early and late onset infections were due to gram positive bacteria (60%), but in contrast to our finding that the highest mortality rate was with gram positive bacteria (64%) Mahmood et al (2002) have found that in case of late onset neonatal sepsis staphylococcus aureus was the predominant agent This was probably acquired from the hospital environment and the attendants. But our isolates of Staphylococcus aureus were methicillin resistant MRSA and were mainly isolated in cases of early onset sepsis (9%).

In this study the mortality in early onset sepsis was 5/33 (15.2 %) and was quite similar to that in late onset sepsis 6/41 (14.6%) (p=0.950). The mortality rate was insignificant in male neonates (p value 0.327) in comparison to female neonates with sepsis. The mortality due to Gram negative sepsis (64%) was significantly more than Gram positive sepsis (36%).This was in agreement with a study in Bangladesh (Hafsa et al., 2011) which showed that mortality rate in EONS (Early Onset Neonatal Sepsis) was significantly higher than LONS (Late Onset Neonatal Sepsis).

Antibiotic resistance among the organisms in the study conducted by Mahmood et al was quite high. More than 90% of the gram-negative rods were resistant to ampicillin (AMP) and co-trimoxazole (COT). Resistance to gentamicin (GEN) was more than 80% and about 70% of these were also resistant to the third generation cephalosporins. More than 90% of GNR were susceptible to meropenem (MEM) and amikacin (AK). A similar high resistance to AMP and the third generation cephalosporins has been reported in a study conducted at Karachi (Anwar et al., 2000). They stated that in case of Staphylococcus aureus all isolates were found resistant to ampicillin while its resistance to methicillin was 61.54%. But majority of these isolates was susceptible to AK and all to vancomycin (VAN) (Mahmood et al., 2000).

In our study gram negative bacteria showed multidrug-resistance pattern, 75% of Acinetobacter spp were Pandrug resistant PDR [defined as Acinetobacter spp resistant to all currently used systemic antimicrobials (Chan et al., 2007)] resistant to Ampicillin 100%, Cotrimoxazole 100%, Amoxicillin/Clavulenic acid 100%, quinolones 93%, Carbapenems 87%, Ceftazidime 87%, Amikacin 80%, Ceferazone- sulbactam 73%. Multidrug resistant Klebsiella species were all resistant to Ampicillin, 3rd generation cephalosporins, Piperacillin-tazobactam, ceferazone-sulbactam, gentamicin and were sensitive to carbapenems 73% and amikacin 67% (Table 3). Gram positive bacteria including MRSA, CONS, Streptococcus viridans were all sensitive to vancomycin (100%) and majority of CONS and MRSA were sensitive to amikacin 75%(Table 2). In this scenario we have to use vancomycin along with amikacin, or one of the carbapenems empirically to cover all the possible bacterial pathogens. This situation is serious as these are the last line antibiotics available with us. If we continue using these, resistance will obviously emerge against these as well.

Acinetobacter was reported to be increase, in NICU patients (Regev et al., 1993, Allen and Hartman, 2000). The resurgence of multi-drug resistant strains is an important issue. Strains resistant to imipenem are reported to reach 12.9% (Ang and Lee, 1992). Aminoglycoside combined with imipenem, a beta-lactamase inhibitor,or quinolones, was synergistic in vitro against such a strain (Marques et al 1997). Multi-drug resistant Acinetobacter was also noted in NICU (Lee et al., 2004). The most alarming Characteristics of Acinetobacter species is the appearance of strains resistant to almost all currently available antimicrobial drugs (Wang et al., 2003; Chan et al., 2007). In the present study Pan-drug resistant Acinetobacter (PDR) showed resistance more than 75% to all available antibiotics, also multidrug resistant klebsiella spp were resistant to almost all available antibiotics except Amikacin (33%) and Imipenem (27%). To prevent such PDR and MDR strains, we should stress more upon preventive measures, so that minimum of our neonates develop sepsis (Mahmood et al., 2002). These preventive measures should focus on recognition of high-risk infant, strict asepsis during labour and early institution of exclusive breast feeding (Bhutta and Yusuf, 1997). Implementing
contact isolation precautions (i.e. strict adherence to hand hygiene protocol before and after nursing care and donning of gowns and gloves before nursing care), cohorting of these cases and assign a specific nurse, and environmental cleaning. Inanimate objects contaminated with blood and body fluids should be cleaned immediately with 0.1% sodium hypochlorite. Incubators and reusable materials should be cleaned and decontaminated thoroughly after discharge of PDR, MDR case (Chan et al., 2007).

The high antibiotic resistance rates found may be associated with the high frequency at which these antimicrobial drugs were used for both prophylactic and therapeutic treatment of hospitalized newborns. This practice may have exerted selective pressures leading to the emergence of multi-drug resistant strains (Marcio et al., 2002), which in turn may have stimulated the acquisition of genes encoding resistance mechanisms via horizontal transfer mechanisms between bacterial strains within the hospital environment (Marcio et al., 2002). It is of particular interest, that in this study all our patients received antimicrobial drugs before positive blood culture presentation (empiric use), due to clinical manifestations suggestive of sepsis.

Hospitals bring together uniquely vulnerable hosts in special units as in NICUs, which, bacteriologically, are very hostile environments, containing a wide selection of pathogenic, antibiotic resistant organisms with which the patient becomes colonized (David, 1998). Invasive procedures, empiric antibiotics, hyperalimentation, prolonged hospitalization, and extremely low birth weight per se, increase the risk of infection. (Fleer, 1983; Sohn, 2001). To control infections, prolonged use of broad-spectrum antibiotics is often encountered, which leads to the resurgence of multidrug-resistant organisms (Sohn, 2001). Therefore, preventive antibiotics should be used as little as possible, while therapeutic antibiotics should be specific and used as short period of time as possible. The combined use of various antibiotics should likewise be judicious. In conditions where in the use of antibiotics is necessary, rotating antibiotic regimens has been suggested and may be away to solve this problem (Quinn and Rodvold, 2000).

Conclusion:

We need to optimize the use of antimicrobial therapy in the hospital, to implement antibiotic policy programs and guide antimicrobial choice in cases when prophylactic or therapeutic therapy must be started before blood culture and antibiogram results are available, due to the clinical conditions of the patients.

The Mortality rate was similar in both early and late onset sepsis which indicate the requirement of intensive prevention and control strategies in both the maternal emergency department and the neonatal intensive care unit.

To control the infections and limit spreading of MDR or PDR organisms we have to emphasise and stress on the prevention control measures as in the application of aseptic techniques, especially hand washing during manipulation of newborns, which may have facilitated cross-infections while in the maternal emergency department and in NICU, also stressing on having adequate number of caring nurses, proper decontamination and sterilization of instruments, proper environmental care. Also electrokaryotyping of the PDR and MDR isolates from the 2 departments is important to detect relatedness and may help to detect the reservoir of the microorganisms and implementation of control measures.

Abbreviations:

VLBW: very low birth weight  NICU: Neonatal Intensive Care Unit
CLSI: Clinical Laboratory Standard Institute  CONS: Coagulase Negative Staphylococci
MRSA: Methicillin Resistant Staphylococcus aureus  TTN: transient tachypnea of newborn
Spp.: species

References


