Original Article

Pathology

Study of Changing Pattern of Bacterial Isolates in Neonatal Sepsis

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ABSTRACT

Objective: To identify the changing pattern of bacterial (aerobic) isolates of neonatal sepsis

Study Design: Observational study

Place and Duration of Study: The study was conducted at the department of Microbiology, Basic Medical Science Institute of JPMC during the period of January 2009 December 2010.

Materials and Methods: The blood samples were taken from babies admitted at National Institute of Child Health. The study included 200 babies between the ages of 1 to 30 days who were presumed to have sepsis on clinical grounds. Neonates who had already been given antibiotics prior to admission and those who had congenital anomaly were excluded. Two hundred (200) blood samples were processed for blood culture. In the laboratory, specimen were inoculated on differential and selective media accordingly. Gram`s staining and biochemical test were performed by standard techniques.

Results: Out of 200 cases 96 had positive blood cultures of which 73 (76.04%) were gram negative and 23 (23.96%) were gram positive bacterial isolates. In this study Enterobacter cloacae was isolated as leading cause of neonatal sepsis in 23 cases followed by Escherichia coli in 17 cases, Klebsiella pneumoniae in 16 cases, Staphylococcus epidermidis in 15 cases, Pseudomonas aeruginosa in 11 cases, Staphylococcus aureus in 10 cases, Citrobacter freundii in 3 cases Neisseria meningitidis in 1 case, Listeria monocytogenes in 1 case, Haemophilus influenzae in 1 case and Non Typhoidal Salmonella in 1 case.

Conclusion: Varying pattern of bacterial isolates causing neonatal sepsis warrants the need for continuous ongoing review of causative organisms. A periodic review of neonatal sepsis is important as it may help in detecting any change in the infecting organism. Therefore, purpose of present study was to describe bacterial isolate from blood culture and to observe the change in bacteriological pattern to provide prompt treatment for neonatal sepsis.

Key Words:, Neonatal, Bacterial Isolates, Sepsis

INTRODUCTION

Neonatal sepsis or sepsis neonatorum is defined as clinical syndrome characterized by systemic signs of infection and bacteremia[1,2]. Sepsis continues to be a leading cause of neonatal mortality and morbidity even in the presence of advanced antimicrobial therapy and supporting care. Bacterial neonatal sepsis is a major problem, as 10% of all neonates admitted in hospitals are treated with antibiotic for suspected sepsis[3]. Despite major advances in neonatology during the past few decades, many infants still develop life threatening infections during the first month of life[4]. Currently neonatal infections are causing more than 1.6 million deaths yearly, sepsis and meningitis are responsible for most of these deaths[5,6]. Overall, incidence of neonatal sepsis is 1-8 /1000 live births in developing world[7] and 1-4/1000 live births in developed countries[8]. World over, two-third of the global neonatal deaths are contributed by just ten countries mostly in Asia (WHO 2006)[9].Pakistan ranks third in neonatal sepsis[10]. Whereas Asian countries, like Thailand and Sri Lanka have managed to reduce their neonatal mortality rate by well-planned protective measures[11].

The infectious agents associated with neonatal sepsis have changed their pattern over 50 years[12]. Changing pattern in the etiological bacteria during past few decades is due to certain factors including development of new antibiotics and their usage. The clustering of sick neonates within relatively small area and the prolonged survival of very low birth weight (VLBW) preterm babies who previously would have died (Rodrigo 2002)[13]. Pathogens responsible for this serious condition, vary with the geographical area (Mahmood et al 2002)[14].

MATERIALS AND METHODS

The study included babies between the ages of 1 to 30 days who were presumed to have sepsis on clinical grounds (fever, tachypneoa ,tachycardia, lethargy, decreased alertness, alter responsiveness and decreased activity). Two hundred (200) blood samples were processed for blood culture. 2ml of blood was collected from a peripheral vein and inoculated into a blood culture bottle containing Trypticase Soya Broth with Sodium Polyanethol Sulfonate. In the laboratory each specimen was incubated aerobically for 7 days. Inoculation on blood agar and MacConkey's agar plates

were made. Sub-culturing was done as per standard procedures. Any growth was subjected for identification by appropriate staining and biochemical tests (sugar fermentations, citrate test, urea test, oxidase test, methyl red, voguesproskauer test, motility test) performed by standard techniques.

RESULTS

Out of 200 cases 96 had positive blood cultures. In this study out of 96 positive cases 73 (76.04%) were gram negative and 23 (23.96%) were gram positive bacterial isolates (table 1 and fig 1).

Table No.1: Distribution of isolates from neonatal sepsis

Total No of Cases	Total Isolated Bacteria	Gram Negative Bacteria	Gram Positive Bacteria
200	96 (48%)	73 (76.04%)	23(23.96%)

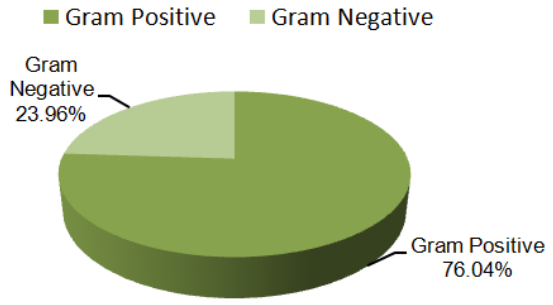

Figure No.1: Distribution of bacterial isolates

Table No.2: Bacteria Isolated from Neonatal Sepsis Blood Cultures (200)

S. No	Bacteria	No of Positive Isolates	Positive Isolates %
1	Enterobacter cloacae	23	23.96
2	Escherichia coli	17	17.71
3	Klebsiella pneumoniae	16	16.67
4	Staphylococcus epidermidis	12	12.50
5	Pseudomonas aeruginosa	11	11.46
6	Staphylococcus aureus	10	10.42
7	Citrobacter freundii	3	3.12
8	Neisseria meningitidis	1	1.04
9	Haemophilus influenzae	1	1.04
10	Non Typhoidal Salmonella	1	1.04
11	Listeria monocytogenes	1	1.04
Total		96	100

In this study Enterobacter cloacae was isolated as leading cause of neonatal sepsis 23 cases followed by Escherichia coli in 17 cases, Klebsiella pneumoniae in 16 cases, Staphylococcus epidermidis in 15 cases, Pseudomonas aeruginosa in 11 cases, Staphylococcus aureus in 10 cases, Citrobacter freundii in 3 cases Neisseria meningitidis in 1 case, Listeria monocytogenes in 1 case, Haemophilus influenzae in 1 case and Non Typhoidal Salmonella in 1 case (table 2).

DISCUSSION

Despite the much more sophisticated diagnosis and management of neonatal sepsis in recent years, it is still a major cause of morbidity and mortality. As pattern of bacteria causing neonatal sepsis differ from place to place and can even change over a period of time[12]..

Organisms isolated during 80s in Lahore (Pakistan) were Escherichia coli, Klebsiella species, Staphylococcus aureus and coagulase-negative Staphylococci in decreasing frequency (Mir et al 1988)[15]. Gram-negative bacteria were related to high morbidity and mortality among newborns in infections with Pseudomonas species (52%) and Streptococcus pneumoniae (100%)[16]. Common organisms isolated in Peshawar were E-Coli (36.6%), Staphylcocus aureus (29.5%), Pseudomonas spp (22.4%), Klebsiella spp (7.6%) and Proteus spp (3.8%). No Group B Streptococcus was isolated (Rahman et al 2001)[17]. In the most recent study National Nosocomial Infections Study (NNIS) data published, Enterobacter is the third and fourth-most-common pathogen recovered from the respiratory tract and intensive care units respectively (Fernandez-Baca 2001; Mahapatra et al 2002)[18,19].

Incidence of early onset sepsis appears to be falling in developed world possibly due to intrapartum antibiotic prophylaxis for group B Streptococci but even then it remains a leading cause of neonatal sepsis in the United States (Watt et al 2001, Hyde et al 2002) [20,21].The increased administration of antenatal ampicillin to pregnant women may be responsible for the increased incidence of early-onset neonatal sepsis with non-group B streptococcal bacteria that are resistant to ampicillin (Towers and Briggs 2002)[22]. Streptoccocus agalactiae is the major cause of neonatal septicaemia which is major health problems in industrailized countries (Isaac2003)[23]. In Malawi the commonest causes of neonatal sepsis were Group B Streptococcus (17%) and non-typhoidal Salmonella (14%) (Milledge 2005)[24]. Most infections are due to Gram-negative pathogens, and many may be environmentally rather than maternally-acquired, owing to unhygienic delivery practices (Zaidi et al 2009)[25].

In our study gram-negative bacteria were isolated in maximum number that is 73 (76.04%) out of 96 positive cases. Among this, majority of the cases were gram-negative bacilli i.e. 72 (98.63%) and only one case of Neisseria meningitides (gram negative diplococcic) was obtained. Most of these cses 60 (83.33%) cases belonged to Enterobacteriaceae.

Majority of the various authors have reported the gram-negative bacterial preponderance, especially Enterobacteriaceae as the cause of neonatal sepsis,[17] In contrast to studies done by Lopez et al [26] and Anwer et al [27] according to which Gram positive organisms were the main cause of neonatal sepsis.

In the present study Enterobacter cloacae has been, found as the leading cause of neonatal sepsis: 23 cases out of total 96 positive cases. This was supported by Fernandez-Baca et al[18;] and Mahapatra et al[19]. Escherichia coli were the second most common bacteria isolated in this study 17 (17.70%). Moreover, it was found as a leading cause of early onset sepsis in neonates.This is in agreement with Alarcon et al [28] who reported Escheriachia coli as the second most common bacteria causing neonatal sepsis and Stoll et al [29] who also mentioned Escherichia coli as second most common cause of early onset sepsis.

Third most common bacterium isolated in this study was Klebsiella pneumonia. It was relatively more common in hospitalized neonates i.e. nosocomial infection Shashikala et al (2000)[30]supported present study as they reported that Klebsiella pneumonia was the most common cause of nosocomial infection in neonates. Staphylococcus was the most frequent gram positive isolate in this study which is similar to other studies of [31,32].In the present study, 11 (11.46%) cases of Pseudomonas aeruginosa were obtained; among these, majority of cases were nosocomial infections i.e. about 9 cases, in which 6 were related to late onset sepsis and 3 cases were related to early onset sepsis. This is in contrast to study done by Dubois et al (2005)[33].One case of each Haemophilus influenza), Non Typhoidal Salmonellae -negative and Listeria monocytogenes (gram-positive rods) were found in this study. Milledge isolated Non Typhoidal Salmonellae as second most common cause of neonatal sepsis[24].

Group B Streptococcus was found not found in our study. In Pakistan studies done by Rahman and Waseem et al[34] and other developing countries[4] are in agreement with present study, they also showed no cases of infection with group B Streptococcus.

CONCLUSION

The study has revealed that bacterial infections are an important problem in the neonatal units with increase rate of mortality and morbidity. There has been a significant change in pattern of pathogens with an increase in gram-negative organisms, particularly the emergence of *E. aerogenes.* Varying pattern of bacterial isolates causing neonatal sepsis warrants the need for continuous ongoing review of causative organisms. A periodic review of neonatal sepsis is important as it may help in detecting any change in the infecting organism. These findings will help in implications for developing prevention and management strategies in communities and hospitals.

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