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Research Paper

Microbiological Profile of Neonatal Sepsis

Medical Science

KEYWORDS : Neonates, sepsis, antimicrobial susceptibility, Bactec

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ABSTRACT

Neonatal sepsis is a major cause of neonatal mortality and morbidity. A prospective study of 150 microbial isolates from blood cultures was done among neonates diagnosed with clinical sepsis. The study involved identification and antimicrobial susceptibility testing of microbial isolates and comaparision of conventional and automated Bactec method of blood culture for diagnosis of sepsis. Escherichia coli and Klebsiella pneumoniae were the predominant microbial isolates and maximum resistance was observed against cephalosporins and aminoglycosides in the present study. The Bactec method recovered significant more organisms than conventional method and cumulative percentage of positivity of 100% was observed 3 days earlier in the Bactec method. Better diagnostic facilities should be used for early detection of sepsis and rational use of antibiotics is recommended.

Introduction:

Every year an estimated 4 million babies die in the first 4 weeks of life. Neonatal sepsis accounts for 26% of the total neonatal deaths[1]. Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection in first month of life[2]. The clinical presentation of neonatal sepsis is non specific and the diagnosis of neonatal sepsis is mainly based on laboratory tests.[3] The gold standard for diagnosis of sepsis is the isolation of the bacteria from a blood culture which takes at least 48 hours to confirm the diagnosis; a delay of which a neonate can ill afford for initiation of appropriate therapy[4].

In developed countries, group B streptococci and coagulase negative *Staphylococci* are the most common aetiologic agents for neonatal sepsis. However, in the developing countries, these organisms are rare with an entirely different bacterial spectrum[5]. The unnecessary exposure to antibiotics in this vulnerable population creates an environment for emergence of bacterial resistance and the potential for poor outcomes.[6]It is therefore critical that sepsis is diagnosed and treated early to improve neonatal outcomes.

With this background, the present study was undertaken to study the microbiological profile, antimicrobial susceptibility pattern and comparision of conventional and automated Bactec method of diagnosis of neonatal sepsis.

Material and Methods:

All neonates diagnosed with clinical sepsis were included in the study during the period from June 2008 to May 2013 in the tertiary care hospital.

Sample collection and processing[7]: Blood sample was collected before starting antibiotic therapy or before the next dose of antibiotics under all aseptic precautions. 2 ml of blood was drawn using a sterile syringe, out of which 1 ml of the blood sample was inoculated aseptically into a blood culture bottle containing 10 ml of Brain heart infusion broth in a ratio of 1:10 and another 1 ml was immediately inoculated into Bactec pediatric bottle for aerobic blood culture. The bottles were immediately brought to the Microbiology laboratory for incubation at 37°C.

Conventional method: Blood culture bottles were carefully examined for macroscopic evidences of growth and gram stained smear was made if the broth showed visible signs of growth. First subculture was made after 18 hours. Thereafter daily subculturing was done for 10 days.

Automated Bactec method: The inoculated Bactec pediatric bottles were incubated for 5 days in the Bactec machine. For those bottles which flagged positive, a Gram stain was performed and an inoculum sub-cultured. If no organisms were seen on Gram stain, after subculture the Bactec bottle was returned to the Bactec machine for further monitoring. Bactec bottles were monitored until the end of the 5 day incubation.

Identification and antimicrobial susceptibility testing was performed for all the bacterial isolates as per CLSI guidelines[8].The data accrued on all neonatal sepsis was analyzed using SPSS version 17.0. A p-value of 0.05 or less was considered statistically significant.

Results:

A total of 470 set of blood cultures were processed by conventional and Bactec method from neonates diagnosed with clinical sepsis.150 positive blood cultures were recovered from the samples.68 isolates were identified by both methods while conventional alone detected 16 isolates and Bactec alone detected 66 isolates.The Bactec method detected significant isolates 89.34% as compared to conventional method which detected 56% only. The Bactec method significantly discovered more organisms as compared to the conventional method with p value less than 0.005.

Table.1 Distribution of organisms recovered from conventional and Bactec method

Organism	Conventional method(%)	Bactec method(%)	Conventional and Bactec method (%)	Total (%)
Gram positive cocci	2	22	19	43
Staphylococcus aureus	2	13	11	26
Coagulase negative Staphylococcus species	0	7	1	8
Enterococcus fecalis	0	1	5	6
Streptococcus pneumoniae	0	1	2	3
Gram negative bacilli	14	44	47	105
Escherichiae coli	8	18	21	47
Klebsiella pneumoniae	5	13	18	36
Pseudomonas aeruginosa	1	9	7	17

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Acinetobacter baumannii	0	2	0	2
Enterobacter aerogenes	0	1	1	2
Citrobacter freundii	0	1	0	1
Fungii	0	0	2	2
Candida species	0	0	2	2
	16(10.67)	66(44)	68(45.34)	150

Table.2 Day wise distribution of organisms detected by conventional method

Organism		Days of organism isolation										
	Total	0	1	2	3	4	5	6	7	8	9	10
Gram positive cocci	21	0	0	1	4	6	5	2	2	1	0	0
Staphylococcus aureus	13	0	0	0	3	4	3	1	1	1	0	0
Coagulase negative Staphylococcus species	1	0	0	1	0	0	0	0	0	0	0	0
Enterococcus fecalis	5	0	0	0	0	1	2	1	1	0	0	0
Streptococcus pneumoniae	2	0	0	0	1	1	0	0	0	0	0	0
Gram negative bacilli	61	0	0	3	7	23	19	9	5	2	0	0
Escherichiae coli	29	0	0	2	3	10	8	3	2	1	0	0
Klebsiella pneumoniae	23	0	0	1	2	8	7	2	2	1	0	0
Pseudomonas aeruginosa	8	0	0	0	2	2	2	1	1	0	0	0
Acinetobacter baumannii	7	0	0	0	0	3	2	2	0	0	0	0
Enterobacter aerogenes	0	0	0	0	0	0	0	0	0	0	0	0
Citrobacter freundii	1	0	0	0	0	0	0	1	0	0	0	0
Fungii	2	0	0	0	0	0	0	0	1	1	0	0
Candida species	2	0	0	0	0	0	0	0	1	1	0	0

Table.3 Day wise distribution of organisms detected by Bactec method

Organism		Days of organism isolation					
	Total	0	1	2	3	4	5
Gram positive cocci	41	0	0	0	11	25	5
Staphylococcus aureus	24	0	0	0	8	14	2
Coagulase negative Staphylococcus species	8	0	0	0	2	5	1
Enterococcus fecalis	6	0	0	0	1	4	1
Streptococcus pneumoniae	3	0	0	0	0	2	1
Gram negative bacilli	91	0	12	33	27	12	7
Escherichiae coli	39	0	6	16	12	3	2
Klebsiella pneumoniae	31	0	4	12	8	4	3
Pseudomonas aeruginosa	16	0	2	4	6	3	1
Acinetobacter baumannii	2	0	0	0	0	1	1
Enterobacter aerogenes	2	0	0	0	1	1	0
Citrobacter freundii	1	0	0	1	0	0	0
Fungii	2	0	0	0	0	1	1
Candida species	2	0	0	0	0	1	1

Table 2 shows that the earliest positivity for conventional method was 2 days while all isolates were identified by 8 days. Table 3 shows that earliest positivity for Bactec method was 1 day while all isolates were identified by 5 days.

43 gram positive organisms, 105 gram negative organisms and 2 Candida species were isolated in present study

Chart 1

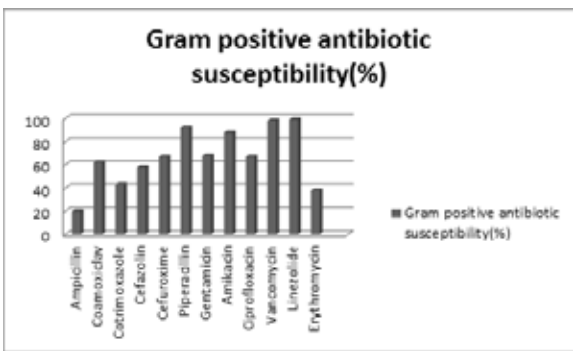
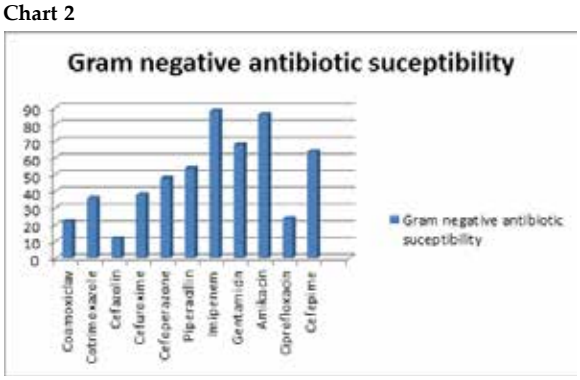


Chart 2



Discussion:

In present study, Gram negative bacilli were found to be the commonest cause of neonatal septicemia (70%) and consisted predominantly of *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa*.. Gram positive organisms were found in 28.67% of total cases and consisted of *Staphylococcus aureus,* Coagulase negative *Staphylococcus species, Enterococcus fecalis* and *Streptococcus pneumonia.* The fetus is frequently exposed to enteric bacteria during the course of maternal peripartal infections. The newborn infant has been shown to have a lack of serum bactericidins against Gram-negative bacilli. Another factor may be the recent widespread use of antibiotics both in the mother and the infant, which would tend to select out the relatively more resistant Gram-negative organisms. Hence; Gram-negative organisms are commonest cause of neonatal septicemia[10].

Among the gram negative isolates, 88 % were sensitive to imipenem and 86% sensitive to amikacin while there was maximum resistance to amoxyclav(22%), cefazolin(12%), cefuroxime(38%) and cefoperazone(48%). The Candida species isolated were sensitive to the antifungals. 74 % of the *Klebsiella* isolates and 83 % of *E. coli* isolates showed ESBL production while there was no ESBL production in *C. freundii* and *E. aerogenes*. The high percentage of ESBL producing *Klebsiella* spp. may be due to selective pressure imposed by extensive use of antimicrobials. 66 % of *Acinetobacter baumannii* and 74% of *Pseudomonas spp.* were sensi-

tive to piperacillin + tazobactum while 72 % of *Acinetobacter baumannii* and 82 % of *Pseudomonas spp*. were sensitive to imipenem, respectively. Three isolates of Pseudomonas and one isolate of *Acinetobacter spp*. were tested for metallo-β-lactamase production. MBL was detected in one isolate of *Pseudomonas aeruginosa* while it was not detected in the isolate of *Acinetobacter baumannii*. 98% of the gram positive organisms were sensitive to vancomycin and linezolid. Methicillin resistance was seen in 2% isolates of *Staph. aureus*. The high resistance rates found may be associated with the frequent use of antimicrobial drugs for both prophylactic and therapeutic treatment of hospitalized newborns[12].

In the present study, isolates were detected 3 days earlier by Bactec than the conventional method.The significance of providing rapid and reliable information to clinicians when a blood culture first becomes positive and reduction in Turnaround time (TAT) to final results has been well documented[9]. Presumptive identification of pathogens are being informed to clinicians hours earlier than a conventional method. This would significantly affect the change of empirical therapy given to patient eventually aiding in the final clinical outcome[11].

Conclusion:

Blood culture with support of automation will be highly useful in the diagnosis of neonatal sepsis. It is possible to provide an etiological diagnosis of the infection with antibiogram within 24 hours of receipt of sample with the use of automation. This helps in targeting the pathogens in a highly specific manner. The time saved will be crucial in saving precious lives of many neonates.

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