Time to Positivity and Antibiotic Sensitivity of Neonatal Blood Cultures

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Abstract

Background: Sepsis is a commonly encountered and potentially life-threatening problem in neonatal intensive care units, blood culture of neonatal sepsis helps in either optimizing treatment or terminating antibiotics. **Materials and Methods:** We determined the causative agent, time to positivity (TTP), and antibiogram of neonatal blood cultures collected in a tertiary care center, to investigate difference between early- and late-onset neonatal sepsis and to establish the time at which a blood culture could safely be considered negative, using the BacT/ALERT® 3D 60. A total of 826 clinically suspected neonates suffering from sepsis and admitted to a neonatal intensive care unit of a tertiary care hospital, Alexandria, Egypt were included in this study. **Results:** Eighty-five (10.29%) showed positive results. The overall TTP median was 21.1 h. Out of the 85 positive cultures, 57 (67.06%) were Gram-positive, 15 (17.65%) were Gram-negative, and 13 (15.29%) were fungi (all *Candida*). Coagulase-negative staphylococci were the predominant organism (41.18%). All the Gram-positive pathogenic isolates were sensitive to vancomycin and tigecycline. Among the Gram-negative isolates, maximum antibiotic sensitivity was observed for levofloxacin. **Conclusion:** We conclude that more than 3 days of incubation may not be required when using the BacT/ALERT® 3D 60 system.

Keywords: Antibiogram, BacT/ALERT, Egypt, neonatal, sepsis

INTRODUCTION

Sepsis is as old as the time of Hippocrates.[1] Neonatal septicemia is a major cause of neonatal mortality aggravated by occurrence of multidrug resistance.[2] It is estimated that about 26% of newborns who die, do so as a result of infections that occur around birth.[3]

Neonatal sepsis can be classified into two subtypes:[4] Early-onset neonatal sepsis (EONS) is commonly considered to be maternally-acquired, mostly caused by *Escherichia coli, Haemophilus influenzae,* and Group B *Streptococcus* (GBS) usually found in the maternal genital tract,[5] whereas late-onset neonatal sepsis (LONS) is considered environmental in origin – either hospital or community acquired. The most common microorganisms implicated in LONS are Coagulase-negative staphylococci (CoNS), *Enterobacteriaceae*, including *Klebsiella pneumoniae* and *E. coli*, and *Acinetobacter baumannii*.[6] In the most recent Eunice Kennedy Shriver National Institute of Child Health and Human Development surveys, LONS was shown to be 10 times more common than EONS in very low birth weight infants.[7]

The isolation of microorganisms from blood is the routine used for diagnosis of sepsis in the newborn infant.[8] The diagnostic potential of blood culture systems has improved over the past decade with the introduction of automated continuous blood culture monitoring systems.[4]

Positive blood cultures help in optimizing treatment, whereas negative cultures aid in early termination of antibiotics. Potential studies on time to positivity (TTP) of blood cultures and duration of antibiotic treatment, especially from developing countries, are scant.[9] There have been recent reports that 5 days of incubation may not be required for some blood culture bottles and some automated blood culture systems.[10-13]

Aim

The objective of the present study was to determine the causative agent, TTP, and antibiogram of neonatal blood cultures collected

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in a tertiary care center and to establish the time at which a blood culture could safely be considered negative.

MATERIALS AND METHODS

The blood cultures were taken from neonates at risk of sepsis or having clinical and/or laboratory indicators of sepsis, between March 2015 and May 2016 in a neonatal intensive care unit of a tertiary care hospital, Alexandria, Egypt, and were prospectively evaluated for 7 days.

The blood culture status, either positive or negative, was recorded till the end of day 7 of incubation. Demographic and laboratory data were collected for all neonates. Neonatal details included gender, age, date and time of birth date (to differentiate suspected EONS and LONS) and time of sample collection, date and time of sample loading, and date and time of signal, if it was detected.

Microbiological methods

The BacT/ALERT® 3D 60 automated blood culture system (BioMérieux, France) was used to process all samples.

One ml venous blood was drawn aseptically and inoculated in BacT ALERT/PF Plus blood culture bottles (BioMerieux, France) with antimicrobial neutralization media and incubated at 37°C aerobically for 7 days.

Growth, if perceived, was then subcultured on MacConkey's agar and 5% sheep blood agar and identified by standard microbiological techniques.[14,15] Using the published Clinical and Laboratory Standards Institute guidelines, the susceptibility profile of the organism tested was then determined.[16]

If there was deteriorating clinical picture or no response to antibiotic therapy over a reasonable period then repeat cultures were performed. Blood cultures were sent to the laboratory 24 h-7 days a week, and the bottle was incubated immediately. The positive blood cultures were differentiated according to the organism isolated into bacterial or fungal. Bacteria were further subdivided into Gram-positive and Gram-negative. Blood cultures with recovery of multiple isolates were excluded from further analysis.

TTP was defined as the time between the beginning of incubation and the time that the signal indicating growth in the culture bottle was detected.

Statistical analysis

The statistical significance of observed differences was evaluated using the Mann–Whitney U-test for non-normally distributed continuous variables and the Chi-square or Fisher's exact test for categorical variables, where appropriate. $P < 0.05$ was considered statistically significant. Data were analyzed with IBM SPSS version 23.0. (Chicago, SPSS Inc.).

RESULTS

A total of 839 blood culture samples from 826 neonates were collected and followed up, and 85 (10.29%) were positive, after excluding repeat cultures.

In suspected EONS, 29 out of 372 (7.8%) blood cultures were positive, whereas in suspected LONS, 56 out of 454 (12.33%) blood cultures were positive (χ^2 = 4.5. P =0.032). Positive cultures isolated from males were 56 (65.88%) and females were 29 (34.12%) (χ^2 = 7.3, P = 0.006) [Table 1].

Out of the 85 positive cultures, 57 (67.06%) were Gram-positive, 15 (17.65%) were Gram-negative, and 13 (15.29%) were fungi (all *Candida*) [Table 1].

CoNS were the predominant organism (41.18%). Other pathogens included *Staphylococcus aureus* (20%), *Candida* (15.29%), GBS and enterococci (both 5.88%), *Klebsiella* and *Acinetobacter* (4.7%), and *E. coli* (2.35%).

Table 2 shows TTP, median TTP, and interquartile range (IQR). The median TTP of all positive blood cultures was 22.1 h (IQR 13.5). A total of 6 (7.1%) blood cultures became positive within the first 12 h after sample incubation, 47 (55.31%) within 24 h and 81 (95.36%) within 48 h. At 72 h of sample incubation, all 85 (100%) blood cultures were positive. Out of the 35 CoNS, only 1 was detected after 48 h. None of the GBS, enterococci, *Klebsiella, E. coli*, and *Acinetobacter* were detected after 48 h.

The median TTP in episodes of suspected EONS was 21.1 h (IQR 17), as compared to 24 h (IQR 13.8) in episodes of suspected LONS (Mann–Whitney U-test = 793.0, P = 0.86). In suspected EONS, only one culture (3.4%) gave a positive signal after 48 h as compared to 3 (5.4%) in suspected LONS.

The median TTP in episodes of suspected EONS and LONS yielding a single Gram-positive isolate were 23.1 h (20.0), as compared to 17 h (IQR12.0) in episodes yielding a single Gram-negative isolate, and 29 h (IQR 15.0) in episodes yielding a single yeast (Kruskal–Wallis χ^2 = 4.461, P = 0.1075).

The median TTP in suspected sepsis episodes yielding a single Gram-positive isolate was longer compared to episodes yielding a single Gram-negative isolate (Mann–Whitney U-test = 427.5, P = 0.496) and shorter compared to episodes yielding a single yeast (Mann–Whitney U-test = 370.5, P = 0.496), whereas the median TTP in suspected sepsis episodes yielding a single Gram-negative isolate was shorter than episodes yielding a single yeast (Mann–Whitney U-test = 97.5, P = 0.492). All 15 Gram-negative isolates were recovered within the first 48 h of sample incubation. Only 2 (3.5%) Gram-positive and 2 (3.5%) yeast isolates were recovered between 48 and 72 h. Non of the isolates were recovered after 72 h [Table 3].

All the Gram-positive pathogenic isolates were sensitive to vancomycin and tigecycline. 91.2% of the isolates were sensitive to cefepime and amoxicillin/clavulanic acid followed by oxacillin (89.5%), imipenem, cefoperazone, and sulfa/ trimethoprim (87.7%), meropenem and ceftriaxone (86%) azithromycin (80.7%), clindamycin (63.2%), amikacin (59.6%), ciprofloxacin (56.1%), ampicillin/sulbactam (50.9%), gentamicin (42.1%), ampicillin (22.8%), and piperacillin (17.5%) [Table 4].

Among the Gram-negative isolates, maximum antibiotic sensitivity was observed for levofloxacin (93.3%), followed

Table 1: Study group characteristics

Characteristic	All (%)	Suspected EOS (%)	Suspected LOS (%)
Total number of blood cultures	839	379 (45.17)	460 (54.83)
Number of neonates	826	372 (45.04)	454 (54.96)
Number of positive blood cultures (before excluding repeats)	98 (11.7)	39 (10.3)	59 (12.83)
Number of positive blood cultures (after excluding repeats)	85 (10.29)	29 (7.8)	56 (12.33)
Number of males	429 (51.94)	192 (51.62)	237 (52.2)
Number of positive culture males	56 (65.88)	19 (65.52)	37 (66.07)
Gram-positive isolates	57 (67.06)	27 (93.1)	30 (53.57)
Gram-negative isolates	15 (17.65)	2 (6.9)	13 (23.21)
Fungi	13 (15.29)	0 (0)	13 (23.21)

EOS: Early-onset sepsis, LOS: Late-onset sepsis

Table 2: Time to positivity of blood cultures according to organism

Characteristic	Number of suspected EOS	Number of suspected LOS	Total (%)	Median	IQR	TTP (h) <12 (%)	12-24 (%)	24-48 (%)	48-72 (%)	>72 (%)
Positive blood cultures	29	56	85	22.1	13.5	6 (7.1)	41 (48.2)	34 (40)	4 (4.7)	0
Staphylococcus aureus	0	17	17 (20)	22	12.5	0	8	8	1	0
Coagulase negative *Staphylococcus*	22	13	35 (41.18)	21	17	1	20	13	1	0
GBS	5	0	5 (5.88)	14.1	13.5	1	3	1	0	0
Enterococci	0	5	5 (5.88)	17	8.5	0	4	1	0	0
Klebsiella	0	4	4 (4.17)	28	29	0	2	2	0	0
Escherichia	2	0	2 (2.35)	24	-	1	0	1	0	0
Acinetobacter	0	4	4 (4.17)	12.2	32.25	2	1	1	0	0
Candida	0	13	13 (15.29)	29	15	1	3	7	2	0

TTP: Time to positivity, GBS: Group B *Streptococcus*, EOS: Early-onset sepsis, LOS: Late-onset sepsis, IQR: Interquartile range

Table 3: Time to positivity of blood cultures according to Gram-staining and onset of sepsis

Characteristic	Median	IQR	<12 (%)	12-24 (%)	24-48 (%)	48-72 (%)	>72 (%)
Suspected EOS	21.1	17.0	2 (6.9)	16 (55.2)	10 (34.5)	1 (3.4)	0
Suspected LOS	24	13.8	4 (7.1)	25 (44.6)	24 (42.9)	3 (5.4)	0
Gram-positive isolates	23.2	12.0	2 (3.5)	31 (54.4)	22 (38.6)	2 (3.5)	0
Gram-negative isolates	17	20.0	3 (20)	7 (46.7)	5 (33.3)	0	0

EOS: Early-onset sepsis, LOS: Late-onset sepsis, IQR: Interquartile range

by imipenem, meropenem, and tigecycline (86.7%), ciprofloxacin (73.3%), tobramycin (60%), ceftriaxone, sulfa/trimethoprim, amoxicillin/clavulanic acid (53.3%), cefoperazone (46.7%), amoxicillin and ofloxacin (20%), gentamicin, and amikacin (13.3%) [Table 5].

DISCUSSION

As high mortality is associated with neonatal sepsis, a fitting antibiotic strategy is needed for its control. The fast identification of microorganisms permits physicians to quickly correct dose, interval or option of antimicrobial therapy while negative cultures help in early discontinuation of antibiotic therapy.[17-19]

We studied a population of neonates dealing with incubation times required for blood cultures to become positive. Our study was conducted to determine the TTP of neonatal blood cultures, to investigate differences between genders, early-onset sepsis (EOS) versus late-onset sepsis (LOS), and to examine differences in TTP by organism type using a prospective observational study, and to determine antibiotic sensitivity patterns.

Our positivity rate (10.29%) compares well with the positivity rate found in most other recent studies.[20-22] Guerti *et al*. noticed a significant difference between the positivity rate of blood cultures collected in infants <72 h of age (3%) and infants ≥72 h of age (33%). This might reflect a less restrictive policy as to the collection of blood cultures in younger infants. Jardine *et al*. reported a total positivity rate of only 6%,[23] whereas Vinod Kumar and Neelagaud reported a positivity rate of more than 50%.[24] The lower positivity rate in the former study might be the result of a higher amount of suspected EOS in this study. The latter study did not report any details that could explain

Table 4: Antibiotic sensitivity patterns among Gram-positive isolates

Antibiotic	Staphylococcus aureus (17), n (%)	Coagulase negative Staphylococcus (35), n (%)	GBS (5), n (%)	Total (57), n (%)
Piperacillin	1 (5.9)	9 (25.7)	0	10 (17.5)
Ampicillin	4 (23.5)	8 (22.9)	1 (20)	13 (22.8)
Oxacillin	17 (100)	30 (85.7)	4 (80)	51 (89.5)
Azithromycin	15 (88.2)	29 (82.9)	2 (40)	46 (80.7)
Clindamycin	6 (35.3)	27 (77.1)	3 (60)	36 (63.2)
Vancomycin	17 (100)	35 (100)	5 (100)	57 (100)
Imipenem	15 (88.2)	30 (85.7)	5 (100)	50 (87.7)
Meropenem	14 (82.4)	30 (85.7)	5 (100)	49 (86)
Ceftriaxone	15 (88.2)	30 (85.7)	4 (80)	49 (86)
Cefoperazone	15 (88.2)	32 (91.4)	3 (60)	50 (87.7)
Cefepime	14 (82.4)	34 (97.1)	4 (80)	52 (91.2)
Tigecycline	17 (100)	35 (100)	5 (100)	57 (100)
Gentamicin	2 (11.8)	21 (60)	1 (20)	24 (42.1)
Amikacin	9 (52.9)	23 (65.7)	2 (40)	34 (59.6)
Ciprofloxacin	5 (29.4)	23 (65.7)	4 (80)	32 (56.1)
Sulfa/trimethoprim	15 (88.2)	31 (88.6)	4 (80)	50 (87.7)
Amoxicillin/clavulanic acid	15 (88.2)	32 (91.4)	5 (100)	52 (91.2)
Ampicillin/sulbactam	6 (35.3)	22 (62.7)	1 (20)	29 (50.9)

GBS: Group B *Streptococcus*

Table 5: Antibiotic sensitivity patterns among Gram-negative isolates

Antibiotic	Enterococci (5), n (%)	Klebsiella (4), n (%)	Escherichia (2), n (%)	Acinetobacter (4), n (%)	Total (15), n (%)
Amoxicillin	2 (40)	1 (25)	0	0	3 (20)
Gentamicin	1 (20)	1 (25)	0	0	2 (13.3)
Amikacin	1 (20)	1 (25)	0	0	2 (13.3)
Tobramycin	4 (80)	3 (75)	1 (50)	1 (25)	9 (60)
Imipenem	5 (100)	4 (100)	1 (50)	3 (75)	13 (86.7)
Meropenem	5 (100)	3 (75)	2 (100)	3 (75)	13 (86.7)
Ceftriaxone	0	3 (75)	2 (100)	3 (75)	8 (53.3)
Cefoperazone	3 (60)	2 (50)	1 (50)	1 (25)	7 (46.7)
Tigecycline	5 (100)	3 (75)	2 (100)	3 (75)	13 (86.7)
Levofloxacin	5 (100)	4 (100)	2 (100)	3 (75)	14 (93.3)
Ciprofloxacin	4 (80)	3 (75)	2 (100)	2 (50)	11 (73.3)
Ofloxacin	2 (40)	1 (25)	0	0	3 (20)
Sulfa/trimethoprim	4 (80)	2 (50)	1 (50)	1 (25)	8 (53.3)
Amoxicillin/clavulanic acid	3 (60)	1 (25)	2 (100)	2 (50)	8 (53.3)

this extremely high positivity rate. These positive cultures should be viewed in light of the immense number of negative cultures resulting every day.

The organisms detected in the present study echo the current spectrum of bacteria and yeast causing neonatal infections that you might find in a developing country. Garcia *et al.*[25] showed that Gram-positive and Gram-negative organisms constituted 80.4% and 10.5%, respectively. In a similar study, *K. pneumoniae*, *S. aureus* and *B. cepacia* were the predominant organisms (68.1%) among definite pathogens.[9] CoNS accounted for 21.6% of total isolates. In 2 Indian studies, *K. pneumoniae* was the most frequently isolated pathogen, followed by *S. aureus* and *E. coli*.[26,27] In contrast, with Garcia *et al.*,[25] *Staphylococcus* accounted for almost 2/3

of the organisms, followed by *Enterococcus*, *Streptococcus agalactiae*, and *E. coli*.

In another study, *Staphylococcus haemolyticus* was the predominant isolate followed by *Staphylococcus epidermidis*. Both this study and Ozkan *et al.* showed that Gram-positive bacterial pathogens were the most common isolates followed by Gram-negative bacterial pathogens and fungi.[27,28]

The EOS was mainly Gram-positive bacteria and most of the Gram–negative. More than half of the Gram-positive, and all *Candida* were LOS.

In several studies, Gram-positive bacterial pathogens were more commonly associated with LOS and Gram-negative pathogens were associated with EOS.[6,27,29]

According to results shown, authors suggest to decrease the antimicrobial spectrum to exclusively target Gram-positive bacteria when the culture is still negative after 48 h and to stop antimicrobial therapy when the culture is still negative after 72 h in clinically well infants.[20,30]

The improvement in blood culture system in the recent years has led to earlier detection of microorganisms. In our study, most of the organisms (96.2% of bacteria and 84.6% of fungi) were detected within the first 48 h of incubation.

This resembles the reports of Garcia et al.[25] (detection of 97.1% of all bacterial pathogens and 88% of fungi within 48 h incubation) and Vamsi et al.[31] (95% of bacteria and 84% of fungi were detected within 48 h of incubation). Janjindamai and Phetpisal[22] also reported the detection of 97% of bacterial pathogens in 75 neonates with suspected sepsis within 48 h.

Vamsi et al. stated that if a blood culture was negative at 36 h, it was 99.14% probable to remain negative.[31] Earlier, Kaiser et al.[32] suggested discontinuation of antibiotics for neonates with possible LOS and negative cultures at 48 h. With a rate of evaluation for suspected sepsis of 20% in neonates[25] and many of them unlikely to have sepsis, shortening the length of antibiotic therapy to 36–48 h was suggested to save significant number of hospital days and doses of antibiotics.

The median TTP in our study was 21.1 h (IQR 13.5). Similar TTP of 21.33 h (Q1–Q3, 13.17–32.46) was reported by Guerti et al.[20] who examined a total of 2916 neonatal blood cultures. Our median TTP was also similar to other studies that used automated blood culture systems.[21,23,33]

In our study, all the Gram-positive pathogenic isolates were sensitive to vancomycin and tigecycline and then came cefepime, amoxicillin/clavulanic acid, oxacillin, imipenem, cefoperazone, sulfa/trimethoprim, meropenem, ceftriaxone, and azithromycin.

Sarangi et al.[27] showed that all their Gram-positive pathogenic isolates were sensitive to linezolid, tigecycline and vancomycin. Co-trimoxazole was sensitive in 78.8% isolates followed by ceftriaxone (77%), azithromycin (76.9%), cefepime (60%), erythromycin (59.6%), and clindamycin (53.9%). This closely resembled the study conducted by Gheibi et al.[34] where maximum sensitivity was found to vancomycin (90%) and ciprofloxacin (78.5%). Study conducted by Katiyar and Bose[35] showed maximum resistance to penicillin (7.41%) and ampicillin (18.52%). Maximum sensitivity to linezolid (100%), vancomycin (95%), cefotaxime (73%), ceftriaxone (68%), and amikacin (68%) was also observed in the study conducted by Mustafa et al.[36]

Among the Gram-negative isolates, in our study, maximum antibiotic sensitivity was observed for levofloxacin, followed by imipenem, meropenem, tigecycline, ciprofloxacin, and tobramycin.

Sarangi et al.[27] showed that maximum sensitivity to amikacin (86.4%) followed by imipenem (77.3%), meropenem (77.3%), tobramycin (77.3%), and ciprofloxacin (68.2%), which was similar to the study conducted by Mustafa et al.[36] In another study conducted by Mane et al.,[37] maximum sensitivity was also found to imipenem (100%), ciprofloxacin (66.6%), and levofloxacin (66.6%), and maximum resistance was found against ampicillin (81.5%) and gentamicin (85.2%).

Our conclusions are restricted by the single center. Our results may not be in harmony with institutions using different laboratory systems because differences in culture media can influence bacterial recovery. A good point in our study is the prospective design permitting for controlling the volume of blood cultures utilized. Similar studies have been carried out with other automated blood culture systems to find out whether a 5-day incubation period is needed for these systems as well. However, unless paired side-by-side studies are performed, cautiousness should be taken when trying to compare the performance of diverse blood culture systems.

CONCLUSION

We have shown that 3 days of incubation may be all that is needed for the detection of bacteria and yeast when using BacT/ALERT® 3D 60 automated blood culture system and BacT ALERT/PF Plus blood culture bottles. Further studies in other institutions are needed to authenticate this study.

This decrease in time will lead to reduction in antibiotic use which may reduce the emergence of resistant organisms, length of hospital stay in a defined neonatal population, and the workload in neonatal unit apart from cost savings. The antibiotic susceptibility profile suggested that for a given cohort the preliminary choice of levofloxacin for Gram-negative bacteria was more rational.

Vancomycin and tigecycline are the drug of choice for Gram-positive organisms.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Angus DC, van der Poll T. Severe sepsis and septic shock. N Engl J Med 2013;369:840-51.
2. Aneja RK, Varughese-Aneja R, Vetterly CG, Carcillo JA. Antibiotic therapy in neonatal and pediatric septic shock. Curr Infect Dis Rep 2011;13:433-41.
3. Guevvera Y. Neonatal and Perinatal Mortality: Country, Regional and Global Estimates. Geneva, Switzerland: WHO Press, World Health Organisation; 2006.
4. Paolucci M, Landini MP, Sambri V. How can the microbiologist help in diagnosing neonatal sepsis? Int J Pediatr 2012;2012:120139.
5. Klinger G, Levy I, Sirota L, Boyko V, Reichman B, Lerner-Geva L; Israel Neonatal Network. Epidemiology and risk factors for early onset sepsis among very-low-birthweight infants. Am J Obstet Gynecol 2009;201:38.e1-6.
6. Jiang JH, Chiu NC, Huang FY, Kao HA, Hsu CH, Hung HY, et al.

Neonatal sepsis in the neonatal Intensive Care Unit: Characteristics of early versus late onset. J Microbiol Immunol Infect 2004;37:301-6.

7. Kaufman D, Fairchild KD. Clinical microbiology of bacterial and fungal sepsis in very-low-birth-weight infants 2004;17:638-80.

8. Saiman L. Infectious diseases of the fetus and newborn infant. JAMA 2012;307:1865-6.

9. Raju VV, Bhat RY, Lewis LE, Vandana KE. Time to positivity of blood cultures in neonates. Pediatr Infect Dis J 2014;33:212-4.

10. Cornish N, Kirkley BA, Easley KA, Washington JA. Reassessment of the incubation time in a controlled clinical comparison of the BacT/Alert aerobic FAN bottle and standard anaerobic bottle used aerobically for the detection of bloodstream infections. Diagn Microbiol Infect Dis 1998;32:1-7.

11. Cornish N, Kirkley BA, Easley KA, Washington JA. Reassessment of the routine anaerobic culture and incubation time in the BacT/Alert FAN blood culture bottles. Diagn Microbiol Infect Dis 1999;35:93-9.

12. Doern GV, Brueggemann AB, Dunne WM, Jenkins SG, Halstead DC, McLaughlin JC. Four-day incubation period for blood culture bottles processed with the Difco ESP blood culture system. J Clin Microbiol 1997;35:1290-2.

13. Han XY, Truant AL. The detection of positive blood cultures by the AccuMed ESP-384 system: The clinical significance of three-day testing. Diagn Microbiol Infect Dis 1999;33:1-6.

14. Collee JG, Marr W. Culture of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons MA, editors. Mackie and McCartney Practical Medical Microbiology. London: Churchill Livingstone; 2006. p. 113-29.

15. Collee JG, Miles RS, Watt B. Tests for identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons MA, editors. Mackie and McCartney Practical Medical Microbiology. London: Churchill Livingstone; 2006. p. 131-49.

16. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. CLSI document M31-A3. 3rd ed. Clinical and Laboratory Standards; 2015.

17. Stoll B. Infections of the neonatal infant–pathogenesis and epidemiology. In: Kliegman RM, Behrman RE, Jenson HB, Stanton BM, editors. Nelson Textbook of Pediatrics. Philadelphia, PA: Saunders; 2008. p. 794-5.

18. Afroza S. Neonatal sepsis – A global problem: An overview. Mymensingh Med J 2006;15:108-14.

19. Hasan AS, Uppal P, Arya S, Capoor MR, Nair D, Chellani H, et al. Comparison of BacT/Alert microbial detection system with conventional blood culture method in neonatal sepsis. J Pediatr Infect Dis 2008;3:21-5.

20. Guerti K, Devos H, Ieven MM, Mahieu LM. Time to positivity of neonatal blood cultures: Fast and furious? J Med Microbiol 2011;60(Pt 4):446-53.

21. Pauli I Jr., Shekhawat P, Kehl S, Sasidharan P. Early detection of bacteremia in the neonatal Intensive Care Unit using the new BACTEC system. J Perinatol 1999;19:127-31.

22. Janjindamai W, Phetpisal S. Time to positivity of blood culture in newborn infants. Southeast Asian J Trop Med Public Health 2006;37:171-6.

23. Jardine L, Davies MW, Faoagali J. Incubation time required for neonatal blood cultures to become positive. J Paediatr Child Health 2006;42:797-802.

24. Vinod Kumar CS, Neelagaud YF. Incubation period for culture positivity to detect septicaemia in neonates. Indian J Med Microbiol 2005;23:270-1.

25. Garcia-Prats JA, Cooper TR, Schneider VF, Stager CE, Hansen TN. Rapid detection of microorganisms in blood cultures of newborn infants utilizing an automated blood culture system. Pediatrics 2000;105(3 Pt 1):523-7.

26. Bhat Y R, Lewis LE, Vandana KE. Bacterial isolates of early-onset neonatal sepsis and their antibiotic susceptibility pattern between 1998 and 2004: An audit from a center in India. Ital J Pediatr 2011;37:32.

27. Sarangi KK, Pattnaik D, Mishra SN, Nayak MK, Jena J. Bacteriological profile and antibiogram of blood culture isolates done by automated culture and sensitivity method in a Neonatal Intensive Care Unit in a tertiary care hospital in Odisha, India. Int J Adv Med 2015;2:387-92.

28. Ozkan H, Cetinkaya M, Koksal N, Celebi S, Hacimustafaoglu M. Culture-proven neonatal sepsis in preterm infants in a neonatal Intensive Care Unit over a 7 year period: Coagulase-negative Staphylococcus as the predominant pathogen. Pediatr Int 2014;56:60-6.

29. Motara F, Ballot DE, Perovic O. Epidemiology of neonatal sepsis at Johannesburg hospital. S Afr J Epidemiol Infect 2005;20:90-3.

30. Bourbeau PP, Pohlman JK. Three days of incubation may be sufficient for routine blood cultures with BacT/Alert FAN blood culture bottles. J Clin Microbiol 2001;39:2079-82.

31. Vamsi SR, Bhat RY, Lewis LE, Vandana KE. Time to positivity of blood cultures in neonates. Pediatr Infect Dis J 2014;33:212-4.

32. Kaiser JR, Cassat JE, Lewno MJ. Should antibiotics be discontinued at 48 hours for negative late-onset sepsis evaluations in the neonatal Intensive Care Unit? J Perinatol 2002;22:445-7.

33. Kumar Y, Qunibi M, Neal TJ, Yoxall CW. Time to positivity of neonatal blood cultures. Arch Dis Child Fetal Neonatal Ed 2001;85:F182-6.

34. Gheibi S, Karamyyar M, Ilkhanizadeh B, Asghari-Sana F, Mahmoodzadeh H, Majlesi AH. Coagulase negative Staphylococcus; The most common cause of neonatal septicemia in Urmia, Iran. Iran J Pediatr 2008;18:237-43.

35. Katiyar R, Bose S. Bacteriological profile of neonatal septicemia in Pravara rural hospital. Pravara Med Rev 2012;4:4-6.

36. Mustafa M, Ahmed SL. Bacteriological profile and antibiotic susceptibility patterns in neonatal septicemia in view of emerging drug resistance. J Med Allied Sci 2014;4:2.

37. Mane AK, Nagdeo NV, Thombare VR. Study of neonatal septicemia in a tertiary care hospital in rural Nagpur. J Recent Adv Appl Sci 2010;25:19-24.