MAR Pediatrics (2023) 4:4

Research Article

Time to Positivity of Blood Culture in Neonatal Sepsis

Moghis Ur Rehman *[1], Hasiat Khan [1], Naveed Ur Rehman [2], Aiman Rahmani [1], Fares Chedid [3]

1. Tawam Hospital, Al Ain, UAE.

2. Sidra Hospital Qatar.

3. Kanad Hospital Al Ain UAE.

*__Correspondence to:__ Moghis Ur Rehman MD, Neonatologist, Tawam Hospital. PO Box (15258) Al Ain, Abu Dhabi, United Arab Emirates.

Copyright

Received: 20 July 2023

Published: 01 August 2023

Abstract

Aim: *To determine the time to positive blood culture in newborn with suspected sepsis and whether it is safe to stop antibiotics by 24-36 hours in asymptomatic babies*

Methods: *A retrospective audit was done of all positive blood culture from neonates between January2010 and December 2014. The bacteremia detection method utilized BacTEC FX system.*

Results: *3148 blood cultures were performed on 17912 newborn during the study period out of which 160 (5.1%) were positive for organisms. One hundred and forty (87.5%) were considered septicemic and twenty (12.5%) were considered contaminants. 67(42%) blood cultures become positive within 12 hours of incubation, 76(47.5%) within 24 hours, 19(11.8%) by 48 hours and only 4 (2.5%) by 72 hours. The median time for gram positive bacteria positivity (definite and possible) was 13.5 and 16 hours with minimum and maximum of 4 to 38 hours respectively. Nine (6.4%) positive blood culture were positive in early onset sepsis (<72hours) while 131 (93.5%) were positive in late onset sepsis (>72hours). All gram negative bacteria median time to positivity was 8 hours with minimum of 1 hour to maximum of 24 hours. Early onset sepsis in our population was caused by gram positive bacteria in 6 out of 9 patients and gram negative in the remaining 3 patients. Thirty one (47.7%) of gram negative infections were SPACE organisms.*

Conclusion: *Time to positive blood cultures significantly differs by organism type. It may be safe to discontinue antibiotics in early onset sepsis and negative coverage for gram negative organism in late onset sepsis by 24hours thus avoiding unnecessary bloods for antibiotic assay.*

Accordingly it may be safe to narrow antimicrobial spectrum to target only gram positive after 24hours and to cease antibiotics after 24hours in early onset Sepsis (EOS) and late onset sepsis (LOS) if blood culture is still negative. Gram negative infections in our setting should be treated with double coverage till the final sensitivity results are available.

Key words: *Blood culture, newborn, septicemia, early onset and late onset sepsis.*

Introduction

Neonatal infection is an important cause of mortality and morbidity in neonatal units, with an incidence of 1 and 4 cases per 1000 live births for full term and preterm babies respectively [1]. The early symptoms of neonatal sepsis are non specific and outlook is considered to be worst in babies in whom antibiotics started late[2], however, and for this reason when the maternal risk factors for neonatal sepsis are present or the infant manifests symptoms suggestive of infection, cultures are obtained and antibiotic therapy is normally initiated[3].

A large number of babies who are evaluated for sepsis don't have proven infection[4, 5]. This means that most of the antibiotics are given to babies without infection in neonatal units setting. Inappropriate use of antibiotics has been implicated in the development of multi-resistant bacteria in hospitals [6]. Because sepsis evaluation and empiric antibiotic therapy are often initiated for subtle indications, it is imperative that antibiotics be stopped in a minimum possible time after the work up for sepsis proves negative. Determination of the appropriate time period in which bacterial cultures can safely be considered negative is the major factor in deciding when to stop antibiotic treatment. Based on these studies many neonatologists are considering to discontinue antibiotic treatment if the blood culture is negative at 48-72 hours and baby does not have any clinical or laboratory indicators of infection [4, 5, 7, 8]. Recently published studies even suggest stopping antibiotic treatment early at 24-36 hours in asymptomatic term babies [9, 10]. These studies have assess the time to positivity of blood culture using modern closed computer based system which detect changes in CO2 indicating growth every 10-15minutes (BacTEC Fx). The BacTEC Fx method is a superior method in detection of paediatric and newborn blood culture than any other method[11].

The primary aim of the present study was to measure the time taken for bacteria to be detected in blood cultures taken from babies in neonatal ICU using the BacTEC/FX microbial detection system. The secondary aim was to define early versus late onset sepsis, true versus contaminant, differences in time to positive blood culture by organism and whether we can stop antibiotics by 24-36 hours in clinically well babies and avoid unnecessary blood sampling for drug level.

Methods

Patients and settings.

A retrospective audit of all positive blood cultures collected from neonates in neonatal intensive care unit (NICU) at Tawam hospital between 1st january 2010 and 31st December 2014.The Neonatal ICU of Tawam Hospital is 52 bed level III care with annual admission rate of approximately 500 neonates. The bacteremia detection method used was the BacTAlert system with Peds bottles.

Definitions

Suspected early onset sepsis was defined as a) onset within 72hrs after birth b) clinical or laboratory evidence of infection c) treatment with broad-spectrum antibiotics started after collecting blood culture.

Late onset sepsis was similarly defined except for the onset after 72hours of life.

The positive blood culture was defined as growth of organism detected from the time of inoculation of the blood into the culture bottle to the time at which the BacT/Alert machine signaled a positive result. The main outcome was time to detection of positive blood cultures. Time to positivity was defined as the time elapsed between specimen collection and the first recorded positive results in microbiology laboratory.

The positive blood cultures were classified based on the organism isolated as bacterial or fungal. Bacteria were further subdivided into definite pathogens, possible pathogens, and contaminants. Definite pathogens were defined as organisms known to cause disease in newborns, for example Group B streptococcus (GBS), Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Enterococcus species, or Pseudomonas aeruginosa. Possible pathogens were defined as organisms known to cause disease under special circumstances, for example, immunocompromised host, presence of indwelling catheters and these include Coagulase-negative Staphylococci, or α-hemolytic Streptococci. Blood cultures containing two or more organisms were considered pathogens if one of them was a definite or possible pathogens12. Contaminants were defined as organisms that rarely cause disease in the newborn i.e. corynebaterium species and propionibacterium species.

Microbiological culture technique

Blood cultures are collected on all infants who have risk factors for sepsis or clinical signs suggestive of infection whether term or preterm. Skin is prepared with chlorhexidine /alcohol solution or isopropyl alcohol wipe prior to collection. The minimum volume of blood required for collection is 0.5 mL with a maximum of 2 mL; however, actual volumes collected are not recorded. Blood is then inoculated aseptically into a BacT/ Alert aerobic blood culture bottle. Blood culture bottles are immediately transported to our microbiology laboratory which operates 24/7 where these bottles are placed in the BacT/Alert microbial detection machine which constantly agitates the bottles at 350C.

Our hospital has been using the BacTEC 9240/FX microbial detection system since 2003. In the BacTEC 9240/FX system, the substrates in the aerobic culture bottle are in an atmosphere of carbon dioxide at sub atmospheric pressure and are designed for the growth of common aerobic, microaerophilic and fastidious bacteria and common yeasts. The temperature is maintained at 35-37ºC. Each culture bottle contains a colorimetric carbon dioxide sensor to measure microbial growth. Because growth of microorganisms produces carbon dioxide, and with the increase in CO2 the color of the gas permeable sensor changes from green to yellow. The BacTEC system tests for carbon dioxide production every 10 minutes, and data points are plotted. Also as the amount of carbon dioxide dissolved in the culture medium raises, a light emitting diode reflects more light, indicating to the computer that the blood culture is positive. The time required for the blood culture to become positive is recorded in the data management system. In the current study, the time to positivity of a blood culture was taken as the time from inoculation of the blood into the culture bottle to the time at which the BacTEC FX signaled a positive result. The preliminary positive results are immediately communicated to physician on call in Neonatal ICU at any time 24/7.

Bottles with positive signals were immediately removed from the instruments and at any time during 24 hours period and aliquot was taken for gram stain and subculture. Bottles remained incubated for 6days before being declared negative.

Data Collection and analysis

In microbiology laboratory, computerized data records were collected for NICU patients who were screened for either early or late onset sepsis during the study period. These records contained for every sample: patient name, date of birth, date and hour of sample collection, sample loaded, sample detection and isolate(s)

cultured. Using a standardized form, data concerning microorganisms identified, number of hours from inoculation to positive culture, patient age at time of culture, patients' history and clinical presentations, and diagnosis were recorded.

Cultures were excluded from analysis if they were duplicate and if the same organism was cultured from the same patient obtained 2-3 days apart, as it is our practice to repeat blood culture after 48 hours of treatment with antibiotics.

Data was entered and analyzed using SPSS version 19, and diagnostic tests were used to analyze the time to positivity of blood culture. Data and variables were separated into early and late onset sepsis according to organism type, SPACE organism, gram staining, hours at which each organism grew and median time to positive blood culture.

Ethical Approval

Ethical approval was obtained from Al Ain District Human Research Committee, AADHRC). Since the study was retrospective and observational, no parental consent was needed.

Results

Early onset sepsis is classically caused by GBS or gram negative bacteria. EOS in our population was caused by gram positive bacteria in 6 out of 9 patients and gram negative in the remaining 3 patients.

Late onset sepsis is generally caused by gram positive bacteria including CONS, staphylococcus aureus and GBS, a variety of gram negative bacteria and fungus. In our population, gram negative 65 (50%) were slightly more common than gram positive bacteria 63 (47%). Fungal infection was rare 3 (2.3%), partly related to the use of fluconazole prophylaxis in extreme low birth infants (< 1000 g BW) in our unit.

All positive blood cultures for gram negative bacteria (N = 68) in our population returned by 24 hours of life, compared to 91.3% for gram positive bacteria including CONS. Based on these results, considerations should be given to stop Gentamycin at 24 hours in asymptomatic patients obviating the need for Gentamycin level. (This should be in the discussion: The drawback of such recommendation is that a considerable proportion of LOS (38% in our population) is due to staphylococcus species, usually resistant to Oxacillin. In such cases, gentamicin offers a synergistic effect that may be beneficial while waiting for the results of

bacterial sensitivity.)

Thirty-one (47.7%) of gram negative infections were due SPACE organism (Serratia, Pseudomonas, Acinetobacter, Citrobacter and Enterobacter), 20 (31.8%) Klebsiella pneumonia and 14 (21.6%) E coli. Nine (13.9%) of gram negatives were pseudomonas. Based on these results, we recommend treating gram negative bacteria in our setting with double gram negative coverage (gentamycin + ceftazidime) while waiting for the final sensitivity results.

	EOS	**LOS n(%)**	**EOS + LOS n(%)**	**0 - 12 hrs**	**12-24 hrs**	**25-36 hrs**	**36-48 hrs**	**48-72 hrs**
Definite bacteria pathogens	**9**	**81 (61.8)**	**90 (64.2)**					
Gram positive	**6**	**13 (9.9)**	**19 (14.2)**					
GBS	6	1	7	7	-	-	-	-
Staph aureus	0	12	12	2	9	1	-	-
Gram negative	**3**	**65(49.6)**	**68 (48.6)**					
E Coli	1	14	15	10	5	-	-	-
Klebsiella	1	20	21	19	2	-	-	-
Psudomonas	1	8	9	5	4	-	-	-
Enterobacter	-	12	12	7	5	-	-	-
Serratia	-	7	7	5	2	-	-	-
Acinetobacter	-	2	2	2	-	-	-	-
Citrobacter	-	1	1	1	-	-	-	-
Brucella	-	1	1	1	-	-	-	-
Fungus	-	**3 (2.3)**	**3 (2.1)**					
Candida Albicans	-	1	1	0	1	-	-	-
Candida tropicalis	-	2	2	2	-	-	-	-
Possible bacterial pathogens	-	**50 (38.2)**	**50 (35.7)**					
Staph. Epidermidis	-	43	43	6	32	4	1	-
Staph. Haemoliticus	-	1	1	-	1	-	-	-
Staph. Capitis	-	4	4	-	1	3	-	-
Staph. warneri	-	2	2	-	2	-	-	-
Subtotal	9	131	140	67	64	8	1	0
Contaminants	12	8	20	0	12	8	2	4
Total	19	139	160					

Table1: Time to positive blood culture according to the organisms

EOS-early onset sepsis: Sepsis < 72 hrs of life, LOS-late onset sepsis: sepsis ≥ 72 hours of life.

Note: The calculated proportions do not include the contaminants

Rate of positive blood culture in our population is 6%. If definite bacterial pathogens are considered, then sensitivity of detection of bacteraemia or fungaemia by 24hours is ---. If only definite and possible bacterial pathogens are considered i.e. contaminants are reclassified as negatives and fungal cultures are removed from analysis.

Time to positivity of blood culture				
	N	**Mean (SD)**	**Median (P25 - P75)**	**Min, Max**
Gram positive bacteria	69			
Definite pathogens (GBS, staph. Aureus)	19	13.1 (6.1)	13.5 (7.8 – 17.5)	4, 26
Possible pathogen (CONS, staph Haemoliticus, Capitis, Warneri)	50	17.5 (5.7)	16 (13.8 – 22.8)	7, 38
Gram negative	68	9.6 (5.4)	8 (6.1 – 12.1)	1, 24
Fungus	3	10.2 (NA)	9.45 (NA)	4, 17
Contaminants	20	31.9 (22.7)	26 (17 - 36.7)	12, 103

Table 2: Time to positivity of blood culture by categories of organisms

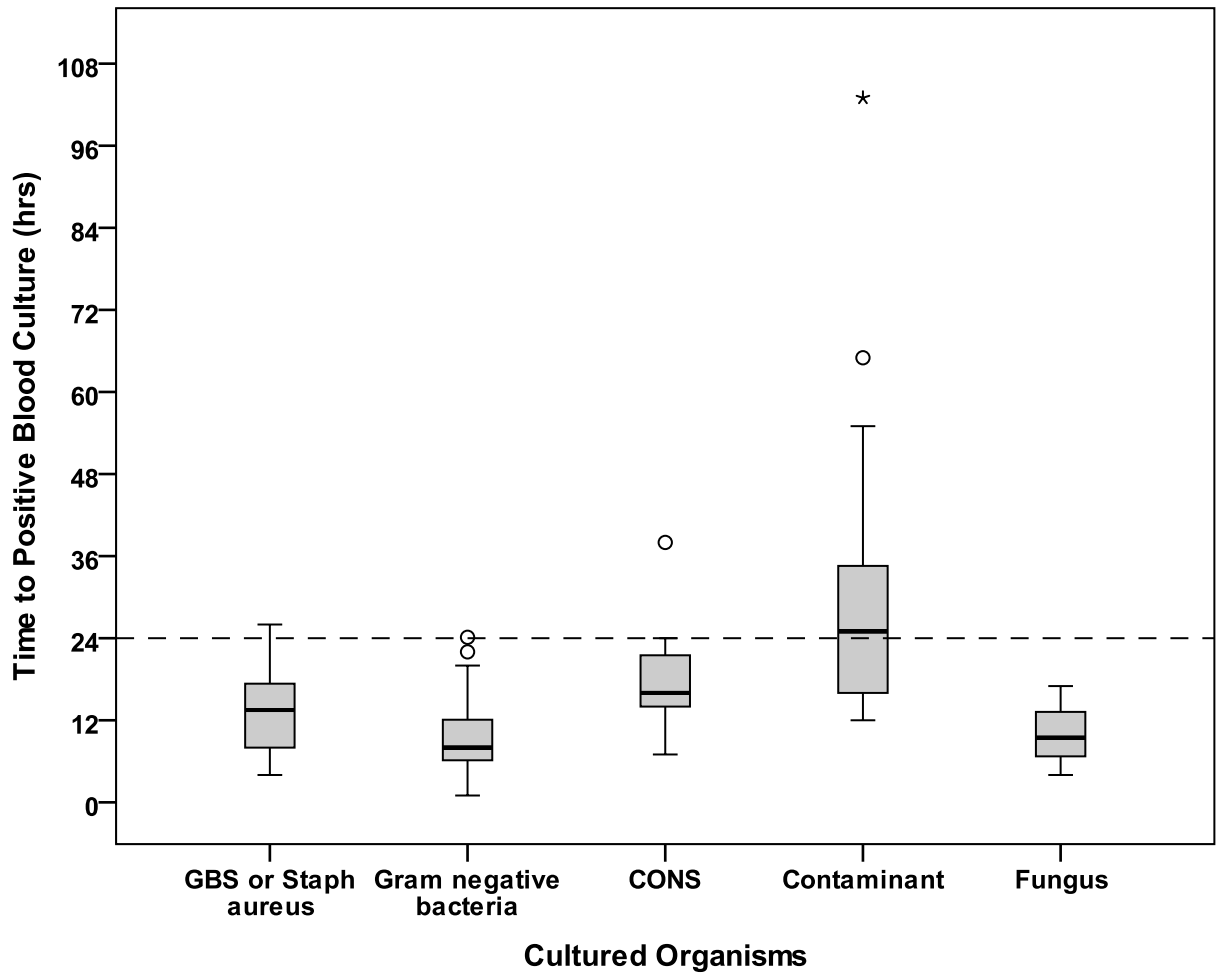

Figure 1: Time to positivity of blood culture by categories of organisms

Discussion

This retrospective analysis of blood cultures of NICU patients at Tawam Hospital using BacTEC 9240/FX system revealed that all positive and gram negative organism in early and late onset sepsis grew within 24hours except one case of staphylococcus aureus in definite bacterial pathogen and 5 cases of staphylococcus epidermidis in possible bacterial pathogens. Early onset sepsis is classically caused by Group B streptococcus (GBS) and gram negative bacteria. In one of the study done for early onset infection screens, only 13 of 413 (3.2%) babies screened for EOS ultimately had any microbiological or clinical and laboratory evidence of infection**[**13**]**. They were all treated with antibiotics for at least 2 days. One

interpretation of this is that >95% of the babies screened and treated for rule out sepsis did not in fact have sepsis and therefore did require antibiotic therapy at all. Since the most common presenting feature of EOS is respiratory, temperature instability and feeding intolerance, the clinician dilemma to differentiate between infectious and non infectious cause is difficult. Improvements in methods to urgently identify or exclude infection help in reducing multiresistant organism from injudicious use of antibiotics as well treating life threatening infections promptly**[**14**]**.

Our data showed a promising results in early onset sepsis (whether gram negative or gram positive organisms) where the time to positive blood culture was less than 24hours. Based on this we can recommend not to measure gentamicin level and to stop antibiotics at 24 hours of life when blood culture is negative at 24 hours provided patient is clinically healthy and having normal values of acute phases reactants (white cell count, absolute neutrophil count, immature to mature ratio and CRP) measured 6 to 12 hours after birth.

Since GBS is the most common organism in EOS, our published data (Ref) 15, also recommend screening with CBC and CRP without blood culture in case of infant born to mother being untreated colonized with GBS. Only when abnormal results are obtained blood should be collected for culture, thus saving cost of length of stay, enhanced mother/infant bonding and encouraging early discharge.

Our results showed that blood culture results after 24 hours for gram positive organisms (N=8) because these organism took longer time for growth while all gram negative organism grew within 24 hours. This is probably due to fact that our microbiology lab works 24/7 with growth being monitored continuously via computerized system. Based on this we are justified in discontinuing gentamicin for gram negative bacteria by 24hours blood culture negative provided there is no evidence of clinical or laboratory infection. This will help to avoid monitoring level and prevent the dosing errors which may occur. Moreover this will also reduce gentamicin induced ototoxicity and sensorineural hearing loss**[**15**]**.

Although gentamicin has some activity against Staphylococcal aureus, flucloxacillin remains the best choice antibiotic for methicillin-sensitive Staphylococcal aureus (MSSA) **[**14**]**.

As with EOS, in situations where clinical improvement is not evident or deterioration is occurring, an empiric change from flucloxacillin to vancomycin or teicoplanin should be considered, together with switching gentamicin for another antibiotic with broader activity against Gram-negative bacteria, for example, piperacillin/tazobactam (tazocin) **[**14**]**.

Thirty one (47.7%) of gram negative infections were due to SPACE (Serratia, Pseudomonas, Acinetobacter, Citrobacter and Enterobacter) organisms.

Our results are comparable for early onset sepsis with other studies1, 5, 11, 16 as all organism grew within 24hours however for late onset sepsis our results differ in that all gram positive organism including CONS grew within 36hours except one Staphylococcus epidermidis which took 48 hours. This can be explained as our microbiology lab operates 24/7 while in luke Jardine16 study there was delay of about 8hours especially when cultures were taken outside the specific time period i.e. 0700 – 2300hours.

The time to positivity in our study is shorter as compared to other studies using modern methods of blood culture incubation techniques **[**9, 10**]**. This is may be because of advances in laboratory methodology and our microbiology laboratory working round the clock and as soon as positive result is signaled, it is instantly conveyed to on call physician and available in clinical record of related infant.

We agree Gracia-Prat et al.**[**9**]** recommendations for ceasing antibiotics in asymptomatic babies by 24-36hours with some modifications to target only gram positive bacteria beyond 24hours if the culture is still negative in LOS and cease antibiotics by 48hours. For EOS it is reasonable to stop antibiotics by 24hours provided antenatal, clinical and laboratory parameters don't point towards infection. We also recommend covering all gram negative blood culture results preliminary with double antibiotics targeting gram negative bacteria as about 50% of our population gram negative sepsis is SPACE organisms.

Our recommendations of early cessation of gram negative antimicrobials and targeting only gram positive after 24hours blood culture negative in asymptomatic infants will results potential benefits in clinical situation. This may result in significant cost savings in length of stay, equipment used, antibiotics given and measuring their assay. This also helps in reducing length of admission time, enhanced mother/infant bonding and a reduction in the emergence of antibiotic resistant bacteria colonising the patients and the environment. If antibiotics were stopped by 24hours in asymptomatic babies with negative blood culture, the babies would suffer less painful procedures like insertion of intravenous canulae for antibiotics administration and blood tests such as antibiotic assays.

The study is limited by its retrospective nature and small sample size. Another limitation is the uncertainty of blood volume inoculated in the culture bottles.

Conclusion

Clinical status of the neonate remains the most important factor in deciding the management of neonatal sepsis and symptomatic neonate will continue to get antibiotics even in the absence of laboratory support, for duration, if the clinical condition so demands. In asymptomatic neonates, there is need to have to have more clear guidelines about discontinuing antibiotics once they are started either due to symptomatology or high perinatal risk scores. In this study we recommend that if the infant in <48hours old at the time of blood culture, antibiotics can be discontinued by 24hours for asymptomatic newborns in the absence of any risk factors for infections. For late onset sepsis after 24hours of negative blood culture antibiotics, clinicians should target only gram positive organism without gram negative coverage. The reason for this difference in early versus late groups is predominantly due to large percentage of staphylococci that cause septicemia in late group and their increased median time to positivity. (Table 2). Double gram negative coverage preliminary till blood culture sensitivity for gram negative organisms. With this approach, significant percentage of term babies will be discharged earlier, which results in earlier mother child bonding and less exposure to painful procedures.

Reference

1. Janjindamai W, Phetpisal S. Time to positivity of blood culture in newborn infants. Southeast Asian J Trop Med Public Health. 2006 Jan; 37(1):171-6.

2. Isaacs D, Moxon ER. Neonatal infections **[**preface**]**. Oxford: Butterworth-Heinemann, 1991.

3. Gerdes JS. Clinicopathologic approach to the diagnosis of neonatal sepsis. Clin Perinatol 1991; 18: 361-81.

4. Rowley AH, Wald ER. Incubation period necessary to detect bacteremia in neonates. Pediatr Infect Dis J 1986; 5:590-1.

5. Kurlat I, Stoll BJ, McGowan JE. Time to positivity for detection of bacteremia in neonates. J Clin Microbiol 1989; 27:1068 - 71.

6. Crosson FJ, Moxon ER. Factors influencing kanamycin resistance in gram-negative enteric neonatal sepsis. Pediatrics 1978; 61:478.

7. Pichichero ME, Todd JK.Detection of neonatal bacteremia. J Pediatr 1979; 94:958 - 60.

8. Hurst MK, Yoder BA. Detection of bacteremia in young infants: Is 48 hours adequate? Pediatr Infect Dis J 1995; 14:711- 13.

9. Garcia-Prats JA, Cooper TR, Schneider VF, et al. Rapid detection of microorganisms in blood cultures of newborn infants utilizing an automated blood culture system. Pediatrics 2000; 105:523 - 7.

10. 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.

11. Kumer Y, Qunili M, Neal TJ, Yoxall CW. Time to positivity of neonatal blood culture. Arch Dis Child Fetal Neonatal Ed 2001; 85: 182 - 6.

12. Faix RG, Rovarik SM. Polymicrobial sepsis among intensive care nursery infants. J Perinatol 1989; 9: 131-6.

13. Luck S, Torny M, d'Agapeyeff K, et al. Estimated early-onset group B streptococcal neonatal disease. Lancet 2003; 361:1953- 4.

14. Alison Bedford Russell, Mike Sharland, Paul T Heath. Improving antibiotic prescribing in neonatal units: Time to act. Arch Dis Child Fetal Neonatal Ed 2012; 97:F141–146.

15. Safer Use of Intravenous Gentamicin for Neonates. NPSA/2010/PSA001. 9 February 2010.

16. Luke Jardine, Mark W Davies, Joan Faoagali. Incubation time required for neonatal blood cultures to become positive. Journal of Paediatrics and Child Health 42 (2006) 797 – 802.