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Bacteriological Profiles in Early-Onset-Sepsis (EOS) and Late-Onset-Sepsis (LOS) in Neonates

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Abstract

Aim

This audit is performed to see the bacteriology profiles in early-onset sepsis (EOS) and late-onset sepsis (LOS) to compare the microorganisms identified and the antibiotic sensitivity results against hospitals' guidelines for empiric treatment of early and late-onset sepsis.

Methods

We retrospectively collected the neonate's microbiological data from the laboratory which included the date blood culture (BC) samples were collected, patients' day of life when the samples were collected to determine whether it was EOS or LOS, time to positivity of BC, and antibiotic susceptibility results.

Results

In EOS, most infection was caused by Gram-positive organisms which were 12 out of 14 isolates (85.7%) with GBS as the most common pathogen identified. In LOS, the number of infections caused by Gram-negative organisms, which were 14 of 25 isolates (56%) was higher than those caused by Gram-positive organisms, which were 11 out of 25 isolates (44%). E. coli was identified as the leading pathogen causing BSI. All organisms were sensitive to the antibiotics used according to the protocol.

Conclusion

Escherichia coli was the most common organism and was sensitive to the first-line antibiotics used. Group B Streptococcus is still the main pathogen in EOS. The rate of antibiotic resistance is low. The audit showed the importance of analysing the bacteriological and antibiotic susceptibility pattern to ensure optimal treatments are administered to infants.

Introduction

Neonatal sepsis could end in devastating results if not treated correctly. This audit is done to see the bacteriology profiles in early-onset sepsis (EOS) and late-onset sepsis (LOS) and to compare the microorganisms identified and the antibiotic sensitivity results against the hospitals guidelines for empiric treatment of early- and late-onset sepsis.

Methods

We retrospectively collected microbiological data that included the date blood culture (BC) samples collected while taking note of the patients' day of life on those dates to determine whether it was EOS or LOS, the time to positivity of BC, and antibiotic susceptibility results. All new-borns from 23 weeks gestation with no birth weight (BW) limits, and alive when the BC were being taken were included. The period for this study is from 1 July 2018 to 31 July 2020. The other information included (taken from the electronic medical chart) is the gestational age, sex, birth weight, whether with the confirmed blood culture the infant was diagnosed as primary or secondary bloodstream infection (BSI), and presence or absence of central lines.

The neonatal period begins at birth till the 28 completed days of life[1]. The terminology 'Suspected sepsis' is used because the clinical diagnosis of sepsis in neonates is difficult because many of the signs of sepsis are nonspecific and are observed with other non-infectious conditions[2]. Although a normal physical examination is present [2-5], bacteraemia can occur in the absence of clinical signs [2,6].

Suspected or confirmed EOS in neonates are episodes that occurred between 0 and 72 hours of life[7]. Suspected or confirmed LOS in neonates are episodes that occurred after 72 hours of life[8] up to 3 months of age[9]. We included the first culture taken during the episode of presumed sepsis for analysis and to see whether that first blood culture was significantly positive or not during that episode of presumed sepsis[10].

If a pathogen is grown, e.g. *Staphylococcus aureus,* group B *Streptococcus* or *Escherichia coli,* in BC, a positive BC is defined as significant as the complications and mortality of infection are significant, as shown in previous studies[11-13]. If a single positive BC with skin flora microorganisms e.g. Coagulase Negative *Staphylococci (*CONS*), Micrococcus* and *Corynebacterium* species, this is considered a contaminant and not a significant positive culture. If there are 2 or more positive BC with skin flora such as *CONS*, they are defined as significant, especially if there are central lines and clinical signs/symptoms[14]. In LOS, positive BC will be stratified whether it is community-acquired or healthcare-associated sepsis. Healthcare-associated sepsis is defined as 'sepsis that occurs more than 48 hours after being admitted to the unit when the patient is more than 72 hours of life'[15]. Community-acquired sepsis is defined as 'infection begins within 48 hours after hospital admission in a patient without recently documented connections to healthcare facilities or modalities'[16].

We will state the source of the infection if it is identified in the medical records of those with LOS. If no source is identified, it is defined as primary bloodstream infection (BSI), and if the BSI is secondary to a focus, it is defined as secondary BSI. The Centers for Disease Control and Prevention (CDC) defined 'primary bloodstream infection' as a laboratory confirmed BSI that is not secondary to an infection at another site of the body[17]. Secondary BSI is then defined as a BSI that is believed to be 'seeded from a site-specific infection at another body site'[17]. Central line-associated bloodstream infection (CLABSI) is defined as a 'laboratory-confirmed BSI not related to an infection at another site that develops within 48 hours after the placement or removal of the central line[14].

According to the new ISO 20776-1 standard, these terms are defined as follows:
'Susceptible: A bacterial strain which is inhibited in vitro by a concentration of a drug that is associated with a high likelihood of therapeutic success'[18].
'Resistant: A bacterial strain which is inhibited in vitro by a concentration of a drug that is associated with a high likelihood of therapeutic failure'[18].

Results

Positive cultures

In total, 1859 blood cultures (BC) were taken from July 2018 to July 2020. After reviewing the charts, repeated cultures during a similar presumed sepsis episode are excluded to obtain the total number of first BC samples taken during that period, either the episode of presumed sepsis or BC taken due to the presence of risk factors for infection (such as in asymptomatic infant but had a sibling with a history of invasive neonatal sepsis in EOS). In total, after the exclusion, there were 1771 BC samples taken on different presumed sepsis episodes.

Out of 1771 BC samples, 1476 (83.3%) BC samples were taken for suspected EOS and 295 (16.7%) BC samples were taken for suspected LOS.

Of the 1476 BC samples for suspected EOS, 29 BC had a growth which is only 1.9%. Of the 29 positive BC, 14 (48.3%) of the BC samples were confirmed with BSI (see Table 1) and 15 (51.7%) were contaminants. Of the 14 confirmed BSI, 11 (78.6%) infants underwent lumbar puncture (LP) and cerebrospinal fluid (CSF) sent for culture and only 1 (7.2%) was positive for group B *Streptococcus* (GBS).

Of the 295 BC samples for suspected LOS, 45 of the BC had a growth that is 15.2%. Of the 45 positive BC, 25 (56.8%) of the BC samples were confirmed bloodstream infections (BSI). Of the 25 confirmed BSI, 14 cerebrospinal fluid (CSF) samples sent for culture and there was no growth in the CSF.

There was a higher yield of significant positive BC in LOS compared to EOS, which was 48.2% in EOS and 56.8% in LOS.

Characteristics of infants with confirmed bloodstream infection (BSI)

There were 14 confirmed bloodstream infection (BSI) during EOS. The mean gestation age was 35 weeks (SD+/-5.6) with mean birth weight of 2906 grams (SD+/-1162). Thirteen had no site-specific infection, therefore, they were classified as primary BSI and one had secondary BSI from early necrotizing enterocolitis (NEC).

There were 25 confirmed positive cultures in LOS in this audit and there were 2 babies with separate sepsis episodes requiring septic work. Therefore, in total, there were 23 babies with confirmed BSI during LOS. The mean gestation age was 28 weeks (SD+/-4.1) and the mean birth weight was 1119 grams (SD+/- 894). Two infants were classified as community-acquired sepsis in which one had primary BSI and another secondary BSI (Urinary tract infection). These neonates arrived from home and represented to our unit. These infants would be in isolation before transferring them to tertiary care once stabilized to minimize cross-infectivity. The other 21 were classified as healthcare-associated infections. Of the 21 infants, 9 had primary BSI, 9 had CLABSI, 3 had secondary BSI due to necrotizing enterocolitis (NEC).

Time to positivity of the positive blood culture (BC) in confirmed BSI

The mean time to positivity of positive BC in confirmed EOS BSI was 11.6 hours (SD + / -3.2). The shortest time to positivity (in EOS) was 6 hours, when GBS grew and the longest time to positivity was 18 hours when *Streptococcus pneumoniae grew.* The mean time to positivity of the positive blood culture in confirmed LOS BSI was 13.8 hours (SD+/-7.5). The shortest time to positivity (in LOS) was 8 hours for *E. coli* and the longest time to positivity was 48 hours for CoNS.

Microbiology Characteristics

Table 1: Distribution of the organisms in EOS and LOS.

Isolates	EOS (n=14)	LOS (n=25)
Total Gram-positive organisms	12 (85.7%)	11 (44%)
Coagulase-negative Staphylococci (CoNS)	0	8
Group B Streptococcus	10	1
Staphylococcus aureus	0	2
Streptococcus pneumoniae	1	0
Listeria monocytogenes	1	0
Total Gram-negative organisms	2 (14.3%)	14 (56%)
Escherichia Coli (E. coli)	2	10
Acinetobacter species	0	1
Klebsiella pneumoniae	0	3

Table 1 showed during EOS, most of confirmed BSI was caused by Gram-positive organisms, which were 85.7% compared to LOS, in which a large proportion of infections were caused by Gram-negative organisms (56%). Ten (10) *GBS* infections in EOS were reported out of 14 confirmed BSI.

There were 8 confirmed BSI during LOS caused by CoNS and all EOS CoNS were considered as contaminants. As mentioned in Section 3.2, there was a total of 9 CLABSI during LOS. Two were caused by *Staphylococcus aureus* and all other 7 were CoNS, which constituted 87.5% of CoNS infection during LOS in our cohort. *E. coli* was the main gram-negative organism that caused LOS as shown in Table 1 which was 10 out of 14 followed by *Klebsiella pneumoniae*.

E. coli was identified as the leading pathogen causing BSI which were 12 out of the total 39 confirmed positive cultures (30.8%).

Susceptibility Patterns

Table 2: Antibiotic susceptibility to Gram-positive organisms in EOS.

Antibiotics	Gram-positive organisms		
	Group B *Streptococcus (*GBS*)* (n=10)	*Streptococcus pneumonia* (n=1)	*Listeria monocytogenes* (n=1)
Amoxicillin	10/10	1/1	1/1
Penicillin	10/10	1/1	1/1
Erythromycin	7/10	1/1	1/1
Clindamycin	8/10	Not tested	Not tested.
Vancomycin	10/10	1/1	Not tested.
Cefotaxime	Not all were tested.	1/1	Not tested.
Cefuroxime	Not tested.	1/1	Not tested.

Table 2 showed that all group B *Streptococcus* (GBS) in EOS were susceptible to Penicillin, with some resistance seen with erythromycin and clindamycin. *Streptococcus pneumoniae* and *Listeria monocytogenes* were susceptible to all antibiotics tested.

Two cultures that grow *E Coli* in EOS were highly susceptible to almost all antibiotics tested which included Cefotaxime, Gentamicin, Ciprofloxacin, Meropenem and Amikacin, although one of the two was resistant to Amoxicillin and Co-amoxiclav.

Table 3: Antibiotic susceptibility for Gram-positive organisms in LOS.

Antibiotics	Gram-positive organisms		
	Coagulase Negative Staphylococcus (CoNS) (n=8)	Group B *Streptococcus (GBS*) (n=1)	*Staphylococcus aureus* (n=2)
Amoxicillin	0/8	1/1	0/2
Penicillin	0/8	1/1	0/2
Erythromycin	0/8	1/1	2/2
Clindamycin	0/8	1/1	2/2
Vancomycin	8/8	1/1	2/2
Teicoplanin	7/8	Not tested.	2/2
Rifampicin	8/8	Not tested.	2/2
Linezolid	8/8	1/1	2/2
Flucloxacillin	1/8	Not tested.	2/2
Gentamicin	3/8	Not tested.	2/2
Fusidic acid	5/8	Not tested.	2/2
Ciprofloxacin	5/8	Not tested.	2/2

Table 4: Antibiotics susceptibility for Gram-negative organisms in LOS.

Antibiotics	Gram-negative organisms		
	Escherichia Coli (E Coli) (n = 10, including 1 Extended Spectrum B-Lactamase producer)	*Klebsiella pneumonia* (n=3, including 2 Extended Spectrum B-Lactamase producers)	*Acinetobacter* (n=1)
Amoxicillin	5/10	0/3	Not tested.
Co-amoxiclav	5/10	1/3	Not tested.
Cefuroxime	8/10	1/3	Not tested.
Cefotaxime	9/10	1/3	Not tested.
Ceftazidime	9/10	1/3	1/1
Cefepime	9/10	1/3	Not tested.
Ciprofloxacin	9/10	3/3	Not tested.
Gentamicin	8/10	3/3	1/1
Piperacillin-Tazobactam	9/10	3/3	1/1
Meropenem	10/10	3/3	1/1
Ertapenem	10/10	3/3	Not tested.
Amikacin	9/10	3/3	1/1

Table 3 showed that both *Staphylococcus aureus* grown in blood culture were susceptible to most antibiotics tested against, except Amoxicillin and Penicillin. All GBS was sensitive to Amoxicillin and Penicillin, a similar finding in EOS. All CoNS were susceptible to Vancomycin, Rifampicin, and Linezolid and only 1 is susceptible to Flucloxacillin.

Table 4 showed that the susceptibility to carbapenem-type antibiotics was very high in gram-negative organisms but very low with Amoxicillin. However, this is not clinically significant, as we do not use ampicillin / amoxicillin empirically, except for Listeria. *E. coli* was susceptible to all generations of cephalosporin antibiotics. All *Klebsiella pneumoniae* organisms grown in blood cultures were susceptible to Gentamicin, Piperacillin-Tazobactam and Amikacin.

Discussion

The number of blood cultures (BC) in EOS was higher compared to LOS. On average, there are 8000 infants delivered yearly in our centre. During the audit period, a total of 14 confirmed BSI in EOS reported, which was 0.87 per 1000 live births with a mean gestation of 35 weeks and weight of 2906 grams. A total of 25 confirmed BSI in LOS were reported with a mean gestation of 28 weeks and a weight of 1119 grams. The yield of positive cultures in LOS was higher. All LOS babies were likely to have had symptoms.

Septic tests in EOS were performed on asymptomatic babies with risk factors for infections and some were symptomatic. Infants with LOS were smaller with earlier gestational age; therefore, they needed a longer period of parenteral nutrition, central access (exposing them to CLABSI), length of stay, thus increasing their risk of developing LOS[8,19]. EOS babies are generally not exposed to factors that increase the risk of sepsis. Primary BSI was the most prevalent diagnosis.

In EOS, most of the infection was caused by Gram-positive organisms (85.7%) with GBS as the most common pathogen identified[20]. There were 10 GBS infections in EOS which was 0.5 infection rate per 1000 live births, similar to European and international reports which is 0.3-1/1000 live births[21,22] with 1 detected in CSF. In LOS, the number of infections caused by Gram-negative organisms (56%) was higher than those caused by Gram-positive organisms (44%) in which in previous studies the most predominant organism causing LOS was CoNS followed by Gram-negative organisms[23]. *E. coli* was the most commonly identified gram-negative organism and CoNS was the most common Gram-positive organism, though E. coli was the leading pathogen for neonatal sepsis in our cohort. Three extended-spectrum beta-lactamases (ESBLs) producing *E. coli* (1) and *Klebsiella pneumonia* (2) were detected; none of these infants had been previously detected by rectal swab screening. There were no reports of methicillin-resistant *S. aureus*, vancomycin-resistant enterococci, or carbapenemase-producing enterobacteriales.

The longest time to positivity (TTP) was at 48 hours, which grew CoNS in LOS. The importance of TTP was for the decision about the duration of antibiotics while establishing the diagnosis in the presumed sepsis. The current recommendation is 7 days or longer depending on the clinical picture of the infant[4]. The longest TTP in EOS was 18 hours, thus, according to the NICE guideline, empirical antibiotics are discontinued if BC is negative after 36 hours in EOS[7].

In EOS, the pathogens detected were highly susceptible to Amoxicillin, penicillin, and Gentamicin. Infants are started empirically on Benzylpenicillin and Gentamicin for EOS while waiting for the culture results, which is similar to NICE recommendation[7]. In LOS, almost all Gram-negative organisms (as the leading cause of LOS), were susceptible to Gentamicin. Both *S. aureus* grown were susceptible to Flucloxacillin, but CoNS was generally resistant to Flucloxacillin but susceptible to Vancomycin. Consistent with these findings, in our unit, for suspected LOS, infants are commenced empirically with Gentamicin and Flucloxacillin. We continue to use Flucloxacillin instead of Vancomycin for empiric LOS therapy as CoNS are less virulent than *S. aureus*. However, we have a low threshold to start Vancomycin if central lines are involved or if a positive culture with CoNS is detected in a sick baby. If there is a clinical suspicion of meningitis, Cefotaxime and Gentamicin especially in less than one month old and Amoxicillin too are administered. We do not rely on Gentamicin monotherapy for the empiric treatment of Gram-negative infections if the baby is very sick. The susceptibility of *E. coli* was high to cephalosporins (11/12 were sensitive). However, only one of three *K. pneumoniae* was susceptible to cephalosporins. There was no resistance reported; meropenem is used in patients with sepsis / septic shock, or lack of clinical response to cephalosporins, or if antibiotic resistance is known to narrower-spectrum antibiotics.

This audit showed the importance of analysing the bacteriological and antibiotic susceptibility pattern to ensure optimal treatments are administered to infants and to reduce the risk of emerging resistance.

Declaration of Conflicts of Interest:
The authors confirm that there are no conflicts of interest to declare.

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