Evaluation of culture-proven neonatal sepsis at a tertiary care hospital in Johannesburg, South Africa

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Background. Organisms causing neonatal sepsis differ by region and the organisms causing sepsis change over time in the same area. The antibiotic susceptibility of microorganisms also changes with time, with emergence of multidrug resistant organisms.
Objective. This study aimed to review the causes of neonatal sepsis and antibiotic sensitivity of organisms causing neonatal sepsis at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) neonatal unit over a 12-month period.
Methods. This was a retrospective descriptive study. All blood cultures obtained from the neonatal unit at CMJAH between 1 January 2012 and 31 December 2012 were reviewed. This was followed by a review of the clinical data of patients with a positive culture.
Results. During the period under study, there were 196 patients with blood-culture-proven neonatal sepsis. This gave an incidence of 10.3 per 100 admissions. Late-onset sepsis accounted for 83.7% of cases of neonatal sepsis. The predominant isolates were *Klebsiella pneumoniae* (32.2%), coagulase-negative *Staphylococcus* (23.7%) and methicillin-resistant *Staphylococcus aureus* (13.1%). The majority of the isolated *K. pneumoniae* were extended-spectrum beta-lactamase (ESBL) producing bacteria with resistance to ampicillin and gentamicin.
Conclusion. Neonatal sepsis is a common problem at the CMJAH neonatal unit. There has been an increase in the predominance of Gram-negative microorganisms as a cause of neonatal sepsis in the CMJAH neonatal unit over recent years, with ESBL-producing *K. pneumoniae* and *Acinetobacter baumannii* being the most prevalent Gram-negative causative agents of neonatal sepsis. Coagulase-negative *Staphylococcus* spp. remains an important cause of neonatal sepsis, and is the most prevalent Gram-positive organism isolated from the neonatal unit at CMJAH. Resistance to commonly used antibiotics regimens was noted to be high in the unit.

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Neonatal sepsis is a significant cause of morbidity and mortality in newborns.[1-4] It is the cause of 1.6 million deaths per annum in developing countries.[1] Organisms causing neonatal sepsis differ in different regions and also change with time in the same area.[1,4,5] The antibiotic susceptibility of microorganisms also changes with time, with the emergence of multidrug resistant organisms.[1,4,5] A periodic survey of the causes of sepsis and their antibiotic sensitivity patterns is essential in the design of effective infection control programmes and in guiding empirical antibiotic therapy.[1,4] Factors associated with neonatal sepsis are well described in the literature.[6-8] The aim of this study was to review the causes of neonatal sepsis at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) neonatal unit and to compare these results with those of previous audits conducted in the same unit.

Methods

The research protocol was submitted and approved by the Human Research Ethics Committee of the University of the Witwatersrand (ref. no. M130350) and the NHLS Ethics Committee.

This was a retrospective descriptive study conducted in the neonatal unit at CMJAH, which is a tertiary care institution. The hospital provides secondary- and tertiary-level services and functions as a referral centre for surrounding clinics and hospitals. All blood cultures obtained from the neonatal unit at CMJAH between 1 January 2012 and 31 December 2012 were reviewed. A list of positive blood cultures was obtained from the National Health Laboratory Service (NHLS) computer data warehouse. This was followed by a review of the clinical data of patients with a positive culture, to identify patients with blood-culture-proven neonatal sepsis. The results of the study were compared with two previous similar studies conducted in the unit.

The following definitions were used in the study:

- Neonate was defined as an infant in the first 28 days of life.
- Culture-proven sepsis was defined as a pathogenic organism, either bacterial or fungal, isolated on blood culture with other clinical and laboratory features consistent with infection.
- Laboratory features of sepsis included an abnormal white cell count, reduced platelet count and/or a C-reactive protein (CRP) >10 mg/L. Age-appropriate definitions of low or high white cell count and reduced platelets count were used.

The following organisms were considered to be contaminants and were excluded from analysis: *Micrococcus* spp.; *Bacillus* spp.; *Corynebacterium* spp.; and *Streptococcus viridans*.

Coagulase-negative *Staphylococcus* was considered significant if two blood cultures drawn within 72 hours of each other grew the same organism or if there was a single positive blood culture in association with other laboratory features of sepsis.

When one significant organism was isolated from the same patient within 7 days, this was considered to be a single episode of sepsis.

Sepsis was categorised as early-onset (EOS) or late-onset sepsis (LOS). EOS was defined as sepsis occurring within 72 hours of life and LOS as sepsis occurring after the first 72 hours of life. Patients with bacteraemia, but with no other features suggestive of sepsis, were excluded from analysis. Infants who were in the neonatal unit and developed sepsis beyond 28 days of life were also excluded from the study. Data were entered into a Microsoft Excel spreadsheet for data management and analysed using Statistica (Dell, USA).

Results

There were 1 903 admissions to the neonatal unit during the study period. This number included babies born and babies not born at the CMJAH. The incidence of neonatal sepsis was 10.3 per 100 admissions, which was based on blood culture-positive results for 196 of the neonates. The majority of cases of neonatal sepsis were of late onset and the median (interquartile range (IQR)) age at the onset of neonatal

sepsis was 7 (4 - 14) days. Based on blood culture results, the incidence of EOS was 1.7 per 100 admissions (*n*=33), while the incidence of LOS was 8.6 per 100 admissions (*n*=164), which accounted for 83.7% of cases of neonatal sepsis (Table1).

In total, 236 microorganisms were isolated on blood culture of 196 patients with culture-proven neonatal sepsis during the study period. Of the 196 patients, 7 had two episodes of neonatal sepsis and 4 had three episodes. There were 10 cases of polymicrobial sepsis, i.e. more than one organism was isolated per episode. Gram-negative organisms were the most commonly isolated organisms in cases of culture-proven neonatal sepsis (49.2%; *n*=116/236). Among the cases with Gram-negative organisms, most were due to ESBL *Klebsiella pneumoniae* (65.5%; *n*=76/116), *Acinetobacter baumannii* (17.2%; *n*=20/116) and *Escherichia coli* (10.3%; *n*=12/116). Gram-positive organisms constituted 42.4% (*n*=100/236) of the isolated organisms. Coagulase-negative *Staphylococcus* (56%; *n*=56/100) and methicillin-resistant *Staphylococcus aureus* (31%; *n*=31/100) were the most commonly isolated Gram-positive organisms. Yeasts accounted for 8.5% (*n*=20/236) of the isolated organisms (Table 2).

Table 1. Patient data

Characteristic	All cases of neonatal sepsis (*N*=196), *n* (%)*
Sex	
Male	117 (59.7)
Place of birth	
Inborn	136 (69.4)
Outborn	53 (27.0)
Unknown	7 (3.6)
Maternal HIV status	
Negative	102 (52.0)
Positive	60 (30.6)
Unknown	34 (17.3)
Antenatal booking status	
Yes	119 (60.7)
No	56 (28.6)
Unknown	21 (10.7)
Mode of delivery	
NVD	96 (49.0)
C/S	78 (39.8)
Unknown	22 (11.2)
Birth weight (g), median (IQR)	1 300 (1 000 – 1 720)
Gestational age (weeks), median (IQR)	30 (28 - 32)

NVD = normal vertex delivery; C/S = caesarean section; IQR = interquartile range.
*Unless otherwise specified.

Table 3 shows the patterns of antibiotic susceptibility for the most commonly isolated Gram-negative organisms in our study. Susceptibility results were not available for all the isolated organisms. Overall, there were high levels of resistance to ampicillin (94.6%), gentamicin (86.7%) and ceftazidime (66.1%). *Klebsiella pneumoniae* was mostly sensitive to meropenem (98.4%; *n*=63/64), amikacin (89.4%; *n*=59/66) and piperacillin/tazobactam (70.3%; *n*=45/64). *K. pneumoniae* was resistant to ampicillin, gentamicin and cefotaxime – all of the isolated strains were resistant to ampicillin and 98.4% were resistant to gentamicin and cefotaxime.

Acinetobacter baumannii was mostly sensitive to amikacin (65%; *n*=13/20) and ceftazidime (75%; *n*=15/20), with high levels of resistance to gentamicin (85%) and meropenem (80%). All of the isolated *A. baumannii* were resistant tazobactam/piperacillin. All of the isolated *E. coli* were sensitive to meropenem and cefotaxime, and a number of isolates were also sensitive to amikacin (65%; *n*=13/20) and tazobactam/piperacillin (92.8%; *n*=13/14). Only 57.1% of the *E. coli* isolates were sensitive to gentamicin.

Bacterial isolates in the current study were considerably different from those obtained in a similar study in the same unit in 2002/3 audit, with some similarities to the 2009/2010 audit (Table 4).

Discussion

This study confirmed that pathogens causing neonatal sepsis change over time, and further emphasises the need for ongoing surveillance.[1,4,5] There has been a significant increase in the predominance of Gram-negative microorganisms as a cause of neonatal sepsis in the CMJAH

Table 2. Organisms causing neonatal sepsis

Organism	Neonatal sepsis (*N*=236), *n* (%)	EOS (*N*=39), *n* (%)	LOS (*N*=197), *n* (%)
Gram-positive	100 (42.3)	19 (48.7)	81(41.1)
CONS	56 (23.7)	12 (30.8)	44 (22.3)
MRSA	31 (13.1)	3 (7.7)	28 (14.2)
Enterococcus faecalis	7 (3.0)	2 (5.1)	5 (2.5)
GBS	3 (1.3)	2 (5. 1)	1 (0.5)
Enterococcus faecium	2 (0.8)	0	1 (0.5)
Listeria monocytogenes	1 (0.4)	0	1 (0.5)
Gram-negative	116 (49)	20 (51.3)	96 (48.7)
ESBL-producing *Klebsiella pneumoniae*	76 (32.2)	9 (23)	67 (34.0)
Acinetobacter baumannii	20 (8.5)	7 (17.9)	13 (6.6)
Escherichia coli	14 (5.9)	2 (5.1)	12 (6.1)
Klebsiella oxytoca	2 (0.8)	2 (5.1)	0
Klebsiella pneumoniae	2 (0.8)	0	2 (1.0)
Pseudomonas aeruginosa	1 (0.40)	0	1 (0.5)
Enterobacter cloacae	0	0	1 (0.5)
Fungi	20 (8.4)	0	20 (10.1)
Candida albicans	13 (5.5)	0	13 (6.6)
Candida parapsilosis	5 (2.1)	0	5 (2.5)
Candida glabrata	2 (0.8)	0	2 (1.0)

EOS = early-onset sepsis; LOS = late-onset sepsis; CONS = coagulase-negative *Staphylococcus*; MRSA = methicillin-resistant *Staphylococcus aureus*; GBS = group B *Streptococcus*; ESBL = extended-spectrum beta-lactamase.

neonatal unit during recent years. The high proportion of Gram-negative microorganisms as a cause of neonatal sepsis is similar to the findings of surveillance studies in other developing countries, where Gram-negative organisms were shown to be the predominant causative agents in both EOS and LOS.[1,9]

The most common isolate overall was *K. pneumoniae*, followed by CONS, MRSA and *A. baumanii*. The predominance of *K. pneumoniae* in the current study agrees with several reports from Nigeria and other developing countries.[9] CONS, a Gram-positive organism, was the most common isolate from cases with EOS. CONS has remained an important cause of neonatal sepsis in our unit and accounted for 23.7% of the microorganisms isolated in this study. This was contrary to reports from other developing countries where CONS was usually among the least commonly isolated organisms,[10] however, this may be because CONS is usually excluded from analysis, as it is often considered to be a contaminant, despite the fact that it is a pathogen in neonates, immunocompromised individuals and patients with foreign bodies such as central venous catheter.[10-12] Further evaluations, such as repeat blood cultures, are required to determine the clinical significance of CONS.[11,13] The importance of CONS as a cause of neonatal sepsis has been reported elsewhere in studies in developed countries and developing countries.[11,14] A one-year prospective study in 8 neonatal units in Australia reported that CONS was the most commonly isolated organism.[11]

There has been a significant increase in the proportion of *Klebsiella* spp. isolated from our unit, with the emergence of ESBL-producing *K. pneumoniae*. In 2012, a study by Ballot *et al.*[4] showed that 70.8% of the isolated *K. pneumoniae* were ESBL-producing strains compared with 97.3% in the current study. ESBL-producing isolates tend to be resistant to beta-lactam antibiotics, including third-generation cephalosporins and to other classes of drugs such as aminoglycosides, co-trimoxazole, tetracycline and fluoroquinolones.[15] These organisms pose a major challenge with limited therapeutic options, particularly in resource-challenged countries.[15]

S. aureus and *A. baumannii* are two other organisms that are becoming predominant in the unit. Ballot *et al.*[4] noted the importance of *A. baumannii* as a cause of neonatal sepsis in the unit, when this microorganism accounted for 10% of the bacterial isolates compared with the previous study where this organism was not isolated.[3]

The emergence of resistant organisms causing neonatal sepsis is now a worldwide problem.[1] Reports of multiresistant bacteria causing neonatal sepsis in developing countries are on the increase.[1] The current study also showed increasing resistance to commonly used antibiotics. Most of the isolated *K. pneumoniae* and *A. baumannii* were resistant to ampicillin and gentamicin, which were the first-line antibiotic agents used in EOS. Overall, 94.6% and 86.7%, of the top 3 gram-negative organisms causing sepsis were resistant to ampicillin and gentamicin, respectively. These organisms also showed high resistance against the ceftazidime with an overall resistance of 66.1%, which is a major concern given that 40.9% of organisms isolated in cases of EOS were due to these organisms. Regarding the treatment of LOS, amikacin and tazobactam/piperacillin were used as first-line agents. Most of the

Table 3. Sensitivity of the isolated Gram-negative organisms

	Bacteria									
	Klebsiella pneumoniae			*Acinetobacter baumannii*			*Escherichia coli*			Overall
Antibiotics	**NT**	**RS**	**%**	**NT**	**RS**	**%**	**NT**	**RS**	**%**	**resistance, %**
Ampicillin	64	64	64.0	15	13	86.0	4	11	78.6	94.6
Gentamicin	64	63	98.4	20	17	85.0	14	5	35.7	86.7
Amikacin	66	7	10.6	20	7	35.0	14	2	14.3	16.0
Tazobactam/ piperacillin	64	19	26.7	20	20	100	14	1	7.1	19.0
Meropenem	64	1	1.6	20	16	80.0	14	0	0	17.3
Cefotaxime	64	63	98.4	0	0	0	14	0	0	1.3
Ceftazidime	64	61	95.3	20	5	25.0	14	7	50.0	66.1

NT = number tested; RS = number of resistant strains.

Table 4. Bacterial isolates at Charlotte Maxeke Johannesburg Academic Hospital: 2002 - 2012

Organism	2002 - 2003, *n/N* (%)	2009 - 2010, *n/N* (%)	2012, *n/N* (%)
Gram-positive	79 (68.1)	134 (54.4)	100 (46.3)
CONS	65 (56.0)	62 (22.5)	56 (25.9)
Staphylococcus aureus	4 (3.4)	23 (8.3)	31 (14.3)
Enterococcus faecalis	4 (3.4)	13 (4.7)	7 (3.2)
GBS	2 (1.7)	10 (3.6)	3 (1.4)
Enterococcus faecium	0	11 (4.0)	2 (0.9)
Listeria monocytogenes	0	0	1 (0.5)
Streptococcus viridans	4 (3.4)	15 (6.1)	0
Gram-negative	37 (31.9)	112 (40.5)	116 (53.7)
Klebsiella spp.	12 (10.3)	47 (18.3)	80 (37.0)
Acinetobacter baumannii	0	27 (11.0)	20 (9.2)
Escherichia coli	20 (17.2)	23 (9.3)	14 (6.5)
Pseudomonas aeruginosa	2 (1.7)	4 (1.6)	1 (0.5)
Other Gram-negative organisms	3 (2.6)	11 (4.5)	1 (0.5)

CONS = coagulase-negative *Staphyloccus*; GBS = group B *Streptococcus*.

K. pneumoniae isolates were sensitive to these antibiotics, while *A. baumannii* was mostly sensitive to amikacin and ceftazidime.

Study limitations

There were some limitations, which resulted from the retrospective nature of the study. Study participants were identified based on blood culture results. Due to the retrospective nature of the study, researchers had no control over how the blood cultures were collected. If strict measures to ensure sterility were not employed while taking blood cultures, and if inadequate volumes were taken, the sensitivity and specificity of the blood cultures may have been affected. The susceptibility to antimicrobial agents only looked at commonly used agents and susceptibility results were not available for all of the isolates. A further limitation to this study is that the data were collected 4 years ago and therefore may be considered outdated. However, no recent data have been published from this unit and the change in different organisms isolated between the time period of this study and previously published studies from the same unit remains significant and emphasises the need for periodic surveillance to determine the prevalence of different organisms causing neonatal infections at different times. This information should inform decisions on rational antibiotic therapy.

Conclusion

Neonatal sepsis is a common problem in the CMJAH neonatal unit. There has been an increase in the predominance of Gram-negative microorganisms as a cause of neonatal sepsis in the CMJAH neonatal unit over the years, with ESBL *K. pneumoniae* and *A. baumannii* being the most prevalent isolates. Coagulase-negative *Staphylococcus* remains an important cause of neonatal sepsis, and is the most commonly isolated Gram-positive organism. Resistance to commonly used antibiotic regimens was high. The increase in the predominance of Gram-negative microorganisms, especially resistant organisms, is of serious concern. We recommend that a prospective study be conducted to establish whether this indeed is true and, if so, that changes be made in the first-line regimens for EOS and LOS.

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