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Epidemiology of bacterial bloodstream infections in very low birth weight neonates at Charlotte Maxeke Johannesburg Academic Hospital

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Abstract: *Introduction:* Very low birth weight (VLBW) neonates are at a higher risk of neonatal sepsis because of immature immune systems and prolonged hospitalisation. The pattern of causative pathogens changes with time therefore frequent surveillance remains essential.

Objectives: To review bacterial organisms causing bloodstream infections and their associated antimicrobial susceptibility pattern.

Methods: A retrospective observational study between 1st January 2016 and 31st December 2016 was conducted. The study population included all VLBW neonates with blood culture-proven infection who were admitted to the neonatal unit at Charlotte Maxeke Academic Johannesburg Hospital (CMJAH).

Results: A total of 479 neonates were admitted to the NICU. There were 206 episodes of infection in 173 of the neonates (36.1%); 184 (89.23%) episodes of sepsis were culture proven late-onset sepsis (LONS) and 22 (10.7%) were culture proven early-onset sepsis (EONS). Gram-positive organisms accounted for the majority of isolates (64.1%) with c*oagulase-negative Staphylococci* (CoNS) being the commonest pathogen in EONS at 68% and LONS at 35% respectively. The retrospective nature of the study meant that it was not possible to determine if CoNS were contaminants or pathogens. There was no case of *Streptococcus agalactiae* in the EONS. The number of multidrug-resistant organisms was more common in LONS than EONS with extended beta-lactamase producers in 20% of gram negatives. The majority of *S. aureus* isolated in LONS were methicillin-resistant *Staphylococcus aureus* (MRSA). Accordingly, the overall susceptibility to the first-line antimicrobials is low.

Conclusion: The current first-line therapy does not provide adequate cover. There is poor susceptibility to ampicillin by most pathogens but it remains an antibiotic of choice for EONS. LONS is still more predominant than EONS. Meropenem and vancomycin are suitable options for LONS.

Keywords: Neonate, sepsis, bacterial, very low birth weight, bloodstream infections

Introduction

Sepsis is a major cause of morbidity and mortality in neonates with a higher incidence reported in low-middle income countries (LMIC) than in higher-income countries[1, 2]. In 2019, 47% of deaths in children under the age of five years occurred in the neonatal period and the majority are due to infection[1, 2]. The pattern of causative organisms continually changes, with the increasing prevalence of resistant organisms. It is therefore necessary to regularly review organisms causing neonatal sepsis and their antimicrobial susceptibility to guide appropriate therapy[3].

Neonatal sepsis is defined as a clinical syndrome, manifested by systemic signs of infection with or without an isolated pathogen on culture[4]. Clinical diagnosis is often very difficult because neonates present with nonspecific signs and symptoms[4]. The major clinical features include fever, bradycardia, temperature instability, glucose instability, apnoea attacks, and respiratory distress. In severe cases of sepsis patients will present with signs of

shock mainly skin colour changes and poor perfusion. While laboratory confirmation requires time, there can never be any justification to delay treatment because that will result in avoidable mortality and morbidity. Appropriate empiric antimicrobial is guided by continuous surveillance of pathogens and their susceptibility to antibiotics used[4].

Early-onset neonatal sepsis (EONS) occurs within the first 72 hours of life. Bhat et al reviewed bacterial isolates of EONS in India while Akindoline et al reported on EONS in Nigeria[3, 5]. The pathogens isolated in these two studies were different, therefore highlighting the need to have local current information about pathogens and their antimicrobial susceptibility pattern to guide empiric antibiotic therapy of choice. In general, the organisms causing EONS are usually acquired from the mother before or during delivery, whereas organisms causing late-onset neonatal sepsis are acquired from the environment or health care workers. The pattern of pathogens is changing with more cases of *K. pneumoniae* and *E. coli* being reported with cases of early-onset neonatal sepsis[6, 7]. The rate of *S agalactiae* infections has been substantially reduced with antibiotic-based prevention strategies in resource-rich countries[7].

Late-onset neonatal sepsis (LONS) occurs after the first 72 hours of life until the end of the neonatal period (28 days of life) and is generally associated with different pathogens and different risk factors to that of EONS[8]. Pathogens isolated in LONS are frequently resistant to many antimicrobial agents. The organisms are mainly gram negatives, *Klebsiella pneumoniae,* and *Escherichia. Coli* are some of the pathogens implicated[8].

In South Africa, Ballot et al found that coagulase-negative *staphylococcus* (*CoNS)* was the most common isolate in LONS (19% of all positive culture results)[4]. This was followed by *K. pneumoniae* at 12.1% and *Acinetobacter baumannii* at 10% respectively[3]. In a study done in Taiwan that looked at both EONS and LONS, gram-positive and gram-negative pathogens were isolated with nearly equal frequency[9].

There has been an increase in the number of multidrug-resistant (MDR) pathogens causing neonatal sepsis over time, with extended-spectrum beta-lactamase (ESBL) *Klebsiella species, Acinetobacter baumannii* and carbapenem-resistant Enterobacterales (CRE) becoming increasingly common isolates in the neonatal period [4]. These MDR pathogens are associated with a high mortality rate. The World Health Organisation (WHO) recommends ampicillin (or penicillin; cloxacillin if staphylococcus infection is suspected) plus gentamicin for empiric treatment of neonates with suspected clinical sepsis[9, 10]. The extensive use of antibiotics has resulted in the emergence of resistant bacterial strains such as gentamicin resistant *Klebsiella* species, third-generation cephalosporins resistant gram negatives and *Methicillin-resistant Staphylococcus aureus* (MRSA) 11. The local study by Ballot et al found that 86% of *CoNS* and 69% of *S. aureus* were MRSA, while 65% of *K. pneumoniae* were ESBL producers [4].

Ullah et al in their Pakistan study found imipenem to be effective against all bacterial isolates and remained the drug of choice for the treatment of neonatal sepsis [10, 12].

Therefore, with this context in mind, this study aimed to investigate the epidemiology of bacterial neonatal sepsis (early and late-onset) and the patterns of bacterial pathogens including their antimicrobial susceptibility in very low birth weight infants.

Subjects and methods

This study was a retrospective record review of very low birth weight (VLBW) neonates with bacterial sepsis who were admitted to the neonatal unit at Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg (CMJAH) between 01 January 2016 and 31 December 2016. Only neonates with culture-proven bacterial bloodstream infections were included in our analysis. The exclusion criteria were neonates with unobtainable records and positive cultures with commensal organisms or fungi. The following organisms were regarded as contaminants: *Micrococcus species, Bacillus species and Corynebacterium species* [4].

Study subjects were identified from the hospital laboratory records. Demographic data was obtained by reviewing hospital records, the admission register and the neonatal computer database in the CMJAH unit. Data was collected on discharge / death of each neonate by attending medical staff and entered into a computer database for the purpose of quality control and clinical audit. Data was managed using Research Electronic Data Capture (RedCAP).

Empiric antibiotic therapy for EONS during the study period was ampicillin and gentamicin, while piperacillin/ tazobactam and amikacin or meropenem and vancomycin were used empirically for LOS. Antibiotic therapy and positive cultures were reviewed in consultation with a microbiologist and antibiotics were tailored according to the sensitivity of the organisms cultured.

Preterm and VLBW neonates have immature immune systems and are therefore at increased risk of sepsis [13]. Coagulase-negative Staphylococci have been described as the most common pathogen in high-income countries [14] and, more recently, as the most common isolate in both EONS and LONS in low to middle-income countries (LMICS) [15]. However, a proportion of CONS isolated in blood cultures from sick VLBW neonates may be contaminants 16. In this retrospective review, it was not possible to establish if CoNS isolates indicated clinical infection or contamination, as a result, CoNS isolates were considered to be clinically significant.

Blood culture bottles were incubated in the bacTAlert (bioMerieux, Marcy L-Etoile) automated blood culture system for seven days. Once sufficient growth was obtained, identification and susceptibility testing were performed using the Vitek 2® (bioMerieux, Marcy L-Etoile) automated identification and susceptibility testing system. Clinical Laboratory Standard Institute guidelines (CLSI) for the relevant year were used to interpret the susceptibility results.

Ethics

The study was approved by the Human Research Ethics Committee of the University of the Witwatersrand (Certificate number M170407). Data were de-identified to maintain patient confidentiality. This was a retrospective record review, so the need for individual patient consent was waived.

Statistical analysis

The statistical analysis of the results was performed using SPSS version 24 (IBM). Categorical variables were described as frequencies and percentages. Continuous variables were described using means and standard deviation.

Results

Study population

There was a total of 479 very low birth weight (VLBW) neonates admitted to the neonatal unit over the study period from 01 January - 31 December 2016. One hundred and seventy-three neonates (36.1 %) developed blood culture-proven bacterial sepsis. None of the 173 neonates had missing hospital records. The mean birth weight of neonates with BSI was 1109 grams (SD 236.1grams), the mean gestational age was 29 weeks (SD 2weeks) and the mean duration of stay was 32 days (SD 25days). Additional characteristics of the VLBW neonates are shown in Table 1.

Table 1: Clinical data of very low birth weight babies with culture-proven bacterial sepsis

Characteristics	N (%) 173(100)
Female	78 (45.0)
Male	95(54.9%)
HIV exposure	68 (39.3)
Antenatal care	132 (76.3)
Outcome (Death)	50 (28.9)
Normal vaginal delivery	79 (45.7)
NCPAP	126 (72.8)
IPPV	66 (38.1)
NEC	17 (9.8)

HIV: Human immunodeficiency virus, NCPAP: Nasal continuous positive airway pressure, IPPV: Intermittent positive pressure ventilation, NEC: Necrotizing enterocolitis

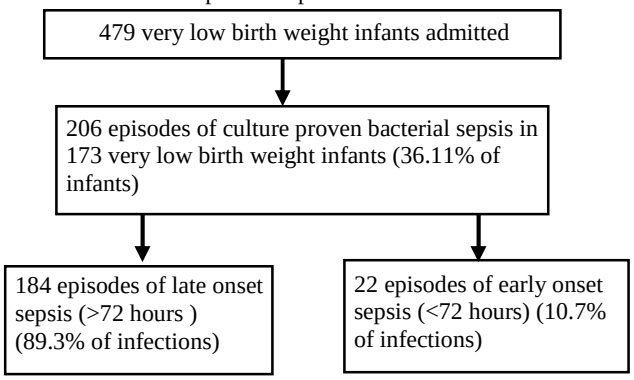
Bloodstream infections

There were two hundred and six episodes of BSI recorded among 173 VLBW neonates; 23 VLBW neonates had multiple episodes of BSI (Table 2). Twenty-two episodes (10.7%) of BSI occurred within the early neonatal period compared to 184 episodes (89.2%) that occurred during the late neonatal period. (Figure 1)

Table 2: Episodes of bacterial bloodstream infection per very low birth weight neonate

Episodes (N)	Neonates (N)
One	150
Two	16
Three	5
Four	1
Five	1

Fig 1: Derivation of the study sample of very low birth weight infants with culture proven sepsis



Pathogens isolated are shown in Table 3.

Table 3: The pathogens isolated in blood during the study period based on gram stain findings

Organisms	EONS N (%)	LONS N(%)
Gram-positive organisms		
Coagulase-negative staphylococcus	15 (68.2)	76(41.3)
Streptococcus agalactiae Staphylococcus aureus	0 2 (9.1)	13(7.1) 10(5.4)
Enterococcus faecalis	0	8(4.3)
Enterococcus faecium	0	6(3.3)
Enterococcus species	0	1(0.5)
Streptococcus viridans	0	1(0.5)
Gram-negative organisms		
Klebsiella pneumoniae	1(4.5)	33(17.9)
Acinetobacter baumannii	3 (13.6)	16(8.7)
Escherichia coli	1(4.5)	12(6.5) 2
Serratia marcescens Pseudomonas aeuriginosa	0 0	(1.1) 1(0.5)
Burkholderia cepacian	0	1(0.5)
Enterobacter aerogenes	0	1(0.5)

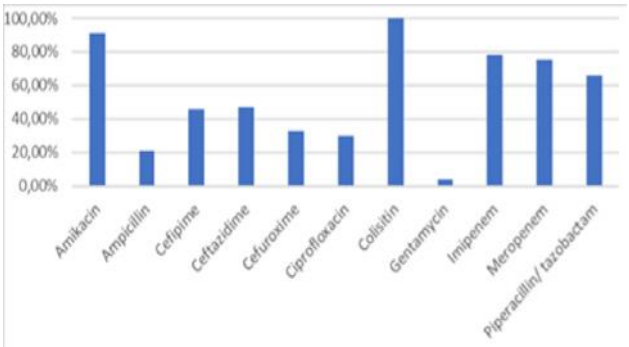
Early-onset neonatal sepsis

There was a total of 22 BSI with a high prevalence of gram-positive organisms (77.3%) as shown in Table 1. There were no cases of *S. agalactiae* isolated during the study period. All five gram negative organisms isolated in EONS were resistant to ampicillin and 2/5 were resistant to gentamicin. Only 10.7% of CoNS were susceptible to first line antibiotics. . The *A. baumannii* infections were all susceptible to colistin but showed a low susceptibility of only 33% to meropenem. There was one case of MRSA infection that was found to be susceptible to vancomycin. The other remaining two isolates were *K. pneumoniae* and *E. coli* infections respectively which were all susceptible to amikacin and meropenem.

Late-onset neonatal sepsis

There were 184 cases of LONS.. The most common isolate was CoNS (41,3%), followed by *K. pneumoniae* (17,9%). Antimicrobial sensitivity of the isolated gram-negative organisms is shown in Figure 2. Of note, the majority of gram-negative organisms were susceptible to empiric LONS antibiotic therapy – namely amikacin (91.3%) meropenem (73.9%) and piperacillin/ tazobactam (66.2%). There were 12.5% (23/184) cases of ESBL infections.

Fig 2: Antimicrobial sensitivity (%) of gram-negative organisms isolated from very low birth weight neonates



There were 5.4% (10/184) cases of LONS due to MRSA and 13/184 cases (7.1%) of *S. agalactaiae,* which were all susceptible to ampicillin/penicillin, and vancomycin.

Discussion

This study showed that more than one-third of VLBW neonates admitted to the study unit had culture-proven bloodstream infections (BSI). Late-onset neonatal sepsis was found to be more prevalent than EONS in a ratio of almost 9:1. These findings are similar to an earlier review conducted in the same unit [4]. VLBW neonates are at increased risk of sepsis because of their underdeveloped immunity, need for central venous access and prolonged hospitalisation [9, 17].

In the present study, the CoNS was the most common isolate in both EONS and LONS. This is in keeping with other reports from LMICS [15] and is similar to findings in earlier studies in the same unit [4, 18]. However, it is difficult to determine which CoNS isolates in ill neonates are actual pathogens as opposed to contaminants [16]. Our findings are in contrast to an Indian study where gram-negative organisms were more prevalent in EONS; *P. aeruginosa* was found to be the commonest pathogen followed by *A. baumannii*. [19]. Recent studies in many LMICS found that in addition to the fact that most of the organisms are gram-negative, there is generally a very low susceptibility to ampicillin [4]. In other studies from LMICS such as Nigeria and India, CoNS was often excluded from the analysis because of difficulty in proving that it is the organism causing the sepsis [19].

There was one case of MRSA in the EONS, which was found to be sensitive to vancomycin. It is worth highlighting that there was no case of ESBL in the EONS. There was a 100% resistance to ampicillin in the EONS group while there was a susceptibility of 71.4% to gentamicin. These findings suggest a review of the first-line empiric antibiotic therapy for EONS, with the possible use of amikacin as a first-line agent for gram-negative organisms.
There were no cases of EONS due to *S. agalactaiae* in the current study. This is in contrast to the previous study in the same unit where seven cases of *S. agalactaiae* were isolated in EONS[4]. However, *S. agalactaiae* was found in 7.1% of isolates in LONS in the current study and all were sensitive to ampicillin. Therefore, ampicillin remains a reasonable choice for antibiotic therapy of *S. agalactaiae*.

The gram-negatives were the predominant isolate in LONS when CoNS is excluded from our analysis. *K. pneumoniae* was the commonest gram-negative accounting for 17.9% of the total infections. There were twenty-four ESBL isolates in the LONS group, which accounted for 13% of total infections. Both the number of *K. pneumoniae* isolates and ESBL producers are more common than in the previous review from the same unit[4]. The *K. pneumoniae* ESBL isolates are intrinsically resistant to ampicillin. The majority were sensitive to piperacillin/tazobactam, amikacin and meropenem, therefore these antibiotics remain a good choice for empiric therapy of LONS. There was a higher proportion of ESBL isolates among *K. pneumoniae* (92%) compared to other gram-negative organisms. The predilection for ESBL production by *K. pneumoniae* has been noted by other researchers previously [11]. The other two cases of ESBL were *S. marcescens* and *Enterobacter spp.* which were both susceptible to amikacin.

There were nine cases of MRSA in the *S. aureus* group. Only one case was susceptible to gentamicin amongst other antibiotics. The majority of *S. aureus* isolated in the LONS group were MRSA. A similarly high prevalence has been reported in other studies in LMIC [6, 20].
It is well known that the risk of nosocomial infection is

increased in prolonged hospitalisation [5, 17]. Infections with *A. baumannii* were also high accounting for 8.7% of the total infections with three infections in the EONS compared to the sixteen in the LONS. It is important to highlight that all *A. baumannii* isolates were in LONS in the previous audit from the same unit[4]. All the isolates were susceptible to colistin followed by ciprofloxacin at 43.7% and low susceptibility to meropenem and cefepime at 18.7%. We did not find any case of pan-resistant *A. baumannii* in our population during the period of the study.

Infections with *E. coli* were the third commonest gram-negative infections accounting for 6.5% of the total infections and all the isolates showed 100% susceptibility to amikacin and cefotaxime. This is similar to the previous report from the same unit [4]. Some researchers have found *E. coli* to be the commonest gram-negative causing neonatal sepsis because of its association with the colonisation of the genital urinary tract system [5]. *Enterococcus* species were also common gram-positive isolates. The susceptibility of *E. faecalis* and *E. faecium* to ampicillin at 76.9%.

The pattern of neonatal sepsis in the CMJAH neonatal unit has changed marginally between 2010 and 2016, with an increase in *K. pneumoniae* ESBL producers. CoNS remains the most common isolate in both EONS and LONS. Of concern is the isolation of resistant gram -negative organisms in EONS. Empiric antibiotic therapy for EONS should possibly be changed to amikacin rather than gentamicin, but other antibiotic protocols remain appropriate.

Conclusion

Neonatal BSI is a common problem at CMJAH neonatal unit. Given the high prevalence of LONS, the importance of strict infection control measures like handwashing and barrier nursing cannot be overemphasised. The antimicrobial susceptibility of pathogens to our first line empiric antibiotics, ampicillin and gentamicin were found to be very low therefore amikacin might be a better aminoglycoside of choice. We recommend that for multi-drug resistant (MDR) gram-negative isolates, meropenem should be initiated while colistin is reserved for resistant *A. baumannii* sepsis. Linezolid and vancomycin are the best options for MDR gram-positive isolates. Ampicillin is still the antibiotic of choice for the treatment of *S. agalactiae* infection. As part of good antibiotic stewardship, it is recommended that antimicrobials should be guided by the blood culture results with susceptibility testing. Because of the findings of the BSI audits in CMJAH neonatal unit, continuous surveillance is strongly recommended to guide the choice of empiric antibiotic therapy.

Study limitations

The study was a retrospective review. There might have been over-reporting in this study due to duplicate isolates. Limited clinical and laboratory data were collected in this retrospective review, so the significance of CoNS isolates could not be determined. Neonatal sepsis was defined by positive blood culture, septic neonates with negative blood cultures were not included in this study. In addition, this report is from 2016 and the organisms causing neonatal sepsis may have changed since then.

Authors' contributions
MM conducted the research for the fulfilment of his MMED degree and collected and analysed the data, wrote various drafts and final submission. VC and DEB supervised MM and assisted in the design of the study, oversight of research, reviewed the various drafts and approved the final draft for submission
Conflict of interests: None
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