Epidemiology of culture-proven early-onset neonatal sepsis in a tertiary hospital in Johannesburg

Tracey Lauren Turner
2067280

Supervisors
Professor Daynia Ballot
Dr Robin Saggers

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in the branch of Paediatrics.

Johannesburg 2021

DECLARATION

I, Tracey Turner, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

20th day of March 2021

ABSTRACT

Background: Neonatal sepsis is poorly defined. The absence of a consensus neonatal-specific definition for sepsis results in variability in reporting early-onset neonatal sepsis and limitations with the comparison of current audits. There is limited research on the epidemiology of early-onset neonatal sepsis in low and middle-income countries.

Methods: To review the epidemiology of culture-proven early-onset neonatal sepsis over six years at Charlotte Maxeke Johannesburg Academic Hospital. This was a retrospective descriptive study of bloodstream culture-proven early-onset neonatal sepsis at Charlotte Maxeke Johannesburg Academic Hospital between 1 January 2013 and 31 December 2018.

Results: There were 100 episodes of early-onset bacterial bloodstream infections over the study period. The majority of infections were Gram-positive (73%). Mortality was significantly higher in Gram-negative sepsis 12/27 (44.4%) as compared with Gram-positive sepsis 9/73 (12.3%) $P < 0.001$. Group B streptococcus culture predominated isolates 37/100 (37%) followed by *Staphylococcus aureus* (15%). *Listeria Monocytogenes* sepsis peaked in 2017, in keeping with the epidemic in South Africa.

Conclusion: Gram-positive organisms continue to be the majority of organisms cultured in newborns with early-onset neonatal sepsis, in the Charlotte Maxeke neonatal unit. Gram-negative sepsis was associated with significantly higher mortality in our study. In order to reach the Sustainable Development Goals and target a neonatal mortality rate below 12 per 1000 by 2030, early-onset neonatal sepsis needs to be addressed at a local and global level.

(Word count 226)

ACKNOWLEDGEMENT

Credit must be given to Professor Daynia Ballot, Doctor Robin Saggers and Rossella Bandini who have patiently guided me through this process.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

CMJAH:	Charlotte Maxeke Johannesburg Academic Hospital
CoNS:	Coagulase-negative staphylococcus
CRP:	C-reactive protein
CS:	Caesarean section
E. coli:	*Escherichia coli*
EONS:	Early-onset neonatal sepsis
GBS:	Group B streptococcus
HIV:	Human Immunodeficiency Virus
IVH:	Intraventricular haemorrhage
LMIC:	Low and middle-income countries
PPIP:	Perinatal Problem Identification Programme
NMR:	Neonatal mortality rate
REDCap:	Research Electronic Data Capture
VGS:	Viridans Group Streptococcus

SUBMISSABLE PAPER

Epidemiology of culture-proven early-onset neonatal sepsis in a tertiary hospital in Johannesburg

T L Turner, MBChB, DCH; **R T Saggers**, MBBCh, FC (Paed), MMed (Paed); **D E Ballot**, MBBCh, FC (Paed), PhD

Department of Paediatrics and Child Health, University of the Witwatersrand, Johannesburg, South Africa

Corresponding author: *T L Turner (traceyturner1986@gmail.com)*

Key Words: Early-onset neonatal sepsis;
Culture–proven;
Epidemiology;
South Africa

ABSTRACT

Background: Neonatal sepsis is poorly defined. The absence of a consensus neonatal-specific definition for sepsis results in variability in reporting early-onset neonatal sepsis and limitations with the comparison of current audits. There is limited research on the epidemiology of early-onset neonatal sepsis in low and middle-income countries.

Methods: To review the epidemiology of culture-proven early-onset neonatal sepsis over six years at Charlotte Maxeke Johannesburg Academic Hospital. This was a retrospective descriptive study of bloodstream culture-proven early-onset neonatal sepsis at Charlotte Maxeke Johannesburg Academic Hospital between 1 January 2013 and 31 December 2018.

Results: There were 100 episodes of early-onset bacterial bloodstream infections over the study period. The majority of infections were Gram-positive (73%). Mortality was significantly higher in Gram-negative sepsis 12/27 (44.4%) as compared with Gram-positive sepsis 9/73 (12.3%) $P < 0.001$. Group B streptococcus culture predominated isolates 37/100 (37%) followed by *Staphylococcus aureus* (15%). *Listeria Monocytogenes* sepsis peaked in 2017, in keeping with the epidemic in South Africa.

Conclusion: Gram-positive organisms continue to be the majority of organisms cultured in newborns with early-onset neonatal sepsis, in the Charlotte Maxeke neonatal unit. Gram-negative sepsis was associated with significantly higher mortality in our study. In order to reach the Sustainable Development Goals and target a neonatal mortality rate below 12 per 1000 by 2030, early-onset neonatal sepsis needs to be addressed at a local and global level.

(Word count 226)

1. Background

Worldwide, an estimated four million children die annually within the neonatal period.(1) Of these newborns, 75% will die within the first week of life with the highest risk of death on the first day of life.(2) The majority of neonatal deaths (99%) occur in low and middle-income countries (LMICs).(2) According to the 2016 Perinatal Problem Identification Programme (PPIP) data, the leading causes of neonatal deaths in South Africa are complications of prematurity, intrapartum-related events, and infections.(3)

Infections continue to cause significant mortality and morbidity in neonatal units across the globe. Incidence and pathogens associated with early-onset neonatal sepsis (EONS) (<72 hours of life) differ widely and change constantly between neonatal units. In LMICs, epidemiology of EONS is largely unknown.(4)

Neonatal sepsis remains poorly defined. The lack of a consensus neonatal-specific definition contributes to current inadequacies in evaluating and comparing neonatal sepsis audits meaningfully.(5) Early clinical identification and diagnosis of neonatal sepsis remains difficult as presentation of sepsis in newborns is non-specific and variable and current markers of early-onset sepsis are neither sensitive nor specific. Delays in initiation of empiric antibiotic treatment results in significant mortality and morbidity. (6) Injudicious use of antibiotics, in neonatal units, has lead to the emergence of antimicrobial-resistant organisms which also adds to neonatal mortality. (6)

Ongoing sepsis audits are essential within neonatal units. They guide programmes designed to reduce the neonatal mortality rate (NMR), aid in the development of vaccines for maternal immunisation against prevalent pathogens like Group B streptococcus and guide empiric antibiotic prescriptions.(7, 8)

This study aimed to review the epidemiology of culture-proven EONS in a tertiary care hospital in Johannesburg over six years.

2. Methods

2.1 Study setting

This was a secondary analysis of an established database reviewing newborns with culture-proven EONS compared to those without EONS at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), a tertiary care academic hospital in Johannesburg, South Africa. CMJAH neonatal unit services approximately 15 000 deliveries per annum – born both in hospital and at satellite midwifery obstetric units, or other hospitals and referred.

Culture-proven EONS was defined as isolation of a pathogenic bacterial organism in the bloodstream of a newborn, before 72 hours of life, associated with clinical signs or laboratory features suggestive of sepsis. Newborns were included if they were inborn and admitted within 72 hours of life to CMJAH neonatal unit between 1st January 2013 and 31st December 2018. Newborns were excluded if: they had missing data about EONS, grew more than one organism on blood culture, or grew an organism considered to be a contaminant on blood culture. Cultures growing *Actinomyces viscosus, Bacillus species*, *Corynebacterium* and *Sphingomonas paucimobilis* were considered contaminants. Coagulase-negative staphylococcus (CoNS) and Viridans Group Streptococci (VGS) were excluded from analysis, as additional data was not available from the database to assign clinical significance of these cultures. Only bacterial bloodstream infections were included in this report.

As a standard of care, all newborns who were assessed by a physician to have a diagnosis of a suspected bacterial infection were admitted to the neonatal unit and were investigated for sepsis. This included: a full blood count, aseptically collected blood culture and a C-reactive protein (CRP) at 24 hours. During the study period, empiric intravenous antibiotics prescribed for all newborns with a suspected early-onset bacterial infection was ampicillin together with gentamicin. Premature newborns presenting with signs of respiratory distress were considered to have possible bacterial infection and

started on the above mentioned empiric antibiotic cover. Antibiotics were discontinued as soon as sepsis was ruled out – that is, the newborn was clinically improving, negative CRP at 24 hours and negative blood cultures at 24 to 48 hours.

2.2 Data collection

This study was a secondary analysis of an existing neonatal database. Data was routinely collected from all newborns admitted to the unit, upon discharge or death, for the purpose of clinical auditing and quality improvement. Data was managed using Research Electronic Data Capture (REDCap) hosted by the University of the Witwatersrand.(9) Data was exported to a Microsoft Excel (Version 14.6.0) spreadsheet. Limited data pertaining to maternal, demographic and clinical characteristics for each patient were included. Data included: maternal age, gravidity, parity, maternal hypertension, mode of delivery, Human immunodeficiency virus (HIV) exposure, chorioamnionitis, presence of culture-proven EONS, birth weight, sex, gestational age, Apgar score at 5 minutes, need for invasive/non-invasive ventilation at delivery, presence of intraventricular haemorrhage (IVH) Grade 3 or 4, length of hospitalization, and outcome at discharge (survived or demised). Chorioamnionitis was based on the maternal records as being present or absent. Grading of IVH was according to Papile.(10) Administration of antenatal steroids was based on maternal records as being administered or not.

2.3 Statistical Analysis

Statistical analysis was done using SPSS version 26 (IBM, USA). Categorical variables were described using frequencies and percentages. Normally distributed continuous variables were described as means and standard deviations, skewed data was described using medians and interquartile range. The group with culture-proven EONS was compared with the group without EONS with regards to maternal, obstetric and clinical characteristics and outcome at discharge using univariate analysis. Chi-square tests were used to compare categorical variables, and continuous variables were compared using unpaired t-tests (for normally distributed variables) and non-parametric tests (for those variables with skewed distribution). Statistically significant differences with a p-value <

0.05 were reported. Cases with missing or unknown values were excluded from the analysis of each variable.

2.4 Ethics

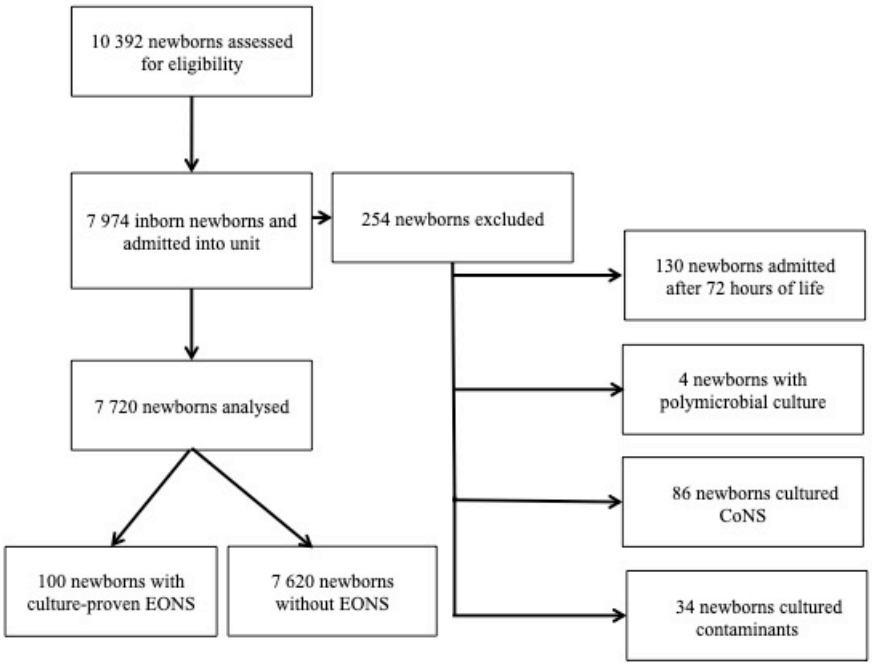
Approval of this study was obtained from the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg (clearance certificate M191153).

3. Results

Figure 1 illustrates how the final sample of 7 720 newborns were selected for inclusion in the study.

Of the 10 398 newborns who were assessed for eligibility 2 418 newborns were excluded as they were either outborn, transferred in from a referral hospital or admitted after 72 hours of life.

Figure 1: Newborns included in the study of culture-proven early-onset neonatal sepsis in a tertiary hospital in Johannesburg, 1 January 2013 to 31 December 2018.



Newborn clinical characteristics

The median birth weight of the newborns was 2010 grams (IQR 1640 grams, n=7719) and the mean gestational age was 34.22 weeks (SD 4.743, n=7712). The majority of admissions were male (n=4151/7698, 53.9%). The majority of newborns were delivered via Caesarean section (CS) (n=4987/7681, 64.9%). The median length of hospital stay was seven days (IQR 17 days, n=6691). HIV exposure occurred in 30% of the newborns (n=2317/7720).

Maternal Characteristics

The mean maternal age was 28.74 years (SD 6.263, n=7355), mean gravidity was 2.46 (SD 1.320, n=7216), and mean parity was 1.2 (SD 1.123, n=7220).

Mortality

Over the study period 11.6 % of the newborns died 893/7719. Twenty-one of the newborns with culture-proven EONS died (n=21/100, 21%). The presence of EONS was strongly associated with mortality (p=0.003).

Bacterial organisms cultured

The organisms causing EONS are tabulated in Table 1. There were 100 episodes of blood culture-proven EONS. The incidence of culture-proven EONS was 1.29 per 100 admissions (100/7720). Gram-positive organisms were isolated in 73 newborns (73%) and Gram-negative organisms in 27 newborns (27%). The most common Gram-positive isolate was Group B streptococcu*s* (GBS) and the most common Gram-negative isolate was *Klebsiella pneumoniae.*

Table 1: Organisms causing early-onset neonatal sepsis in the neonatal unit at a tertiary hospital in Johannesburg, 1 January 2013 to 31 December 2018.

Organism	n (%)
Gram-positive	**73/100**
Staphylococci	
Staphylococcus aureus	15/100 (15)
Enterococci	
Enterococcus faecalis	9/100 (9)
Streptococci	
Group B streptococcus	37/100 (37)
Streptococcus pneumoniae	1/100 (1)
Other	
Listeria monocytogenes	11/100 (11)
Gram-negative	**27/100**
Enterobacteriaceae	
Escherichia coli	9/100 (9)
Citrobacter koseri	1/100 (1)
Klebsiella pneumoniae	10/100 (10)
Carbapenem resistant enterobacteriaceae	1/100 (1)
Other	
Acinetobacter baumannii	3/100 (3)
Haemophilus influenza	2/100 (2)
Pseudomonas aeruginosa	1/100 (1)

In table 2, newborns with culture-proven EONS are categorised into extremely low birth weight (<1000 grams), very low birth weight (1000-1500 grams), low birth weight (1500-2000 grams) and ≥ 2500 grams weight categories.

Table 2: Weight categories of newborns with culture-proven early-onset neonatal sepsis admitted into the neonatal unit, 1 January 2013 to 31 December 2018.

Weight (grams)	Culture-proven EONS n (%)
<1000	17 (17)
1000-1500	19 (19)
1500-2500	30 (30)
≥2500	34 (34)

In Table 3, categorical variables are compared between the group of newborns with culture-proven EONS and those without EONS.

Table 3: Categorical variables of newborns with culture-proven early-onset neonatal sepsis and those without early-onset neonatal sepsis admitted into the neonatal unit, 1 January 2013 to 31 December 2018.

Variable	Culture-proven EONS n (%)	No EONS n (%)	*p*-value *
Vaginal delivery	55/100 (55)	2639/7581 (34.8)	<0.001
Male	52/100 (52)	4099/7598 (53.9)	0.698
HIV exposed	38/100 (38)	2279/7620 (29.9)	0.079
Chorioamnionitis	5/92 (5.4)	233/7245 (3.2)	0.233
Apgar at 5 minutes ≤ 5	13/96 (13.5)	732/7497 (9.8)	0.216
Received antenatal steroids	16/88 (18.2)	1374/6033 (22.8)	0.307
Intraventricular haemorrhage (Grade 3 or 4)	4/17 (23.5)	138/1448 (9.5)	0.052
Maternal hypertension	9/91 (9.9)	1249/7255 (17.2)	0.065
Multiple pregnancy	10/98 (10.2)	923/7563 (12.2)	0.547
Resuscitation at delivery	43/99 (43.4)	3170/7558 (41.9)	0.765
Non-invasive ventilation at birth	39/99 (39.4)	2529/7488 (33.8)	0.240
Invasive ventilation at birth	22/100 (22)	818/7450 (11)	<0.001

**Chi-square*

There were significant associations between vaginal delivery and culture-proven EONS as well as invasive ventilation at birth and culture-proven EONS. There was an association, although not statistically significant, between grade 3 and 4 IVH and EONS. All other clinical variables were not statistically significantly associated with culture-proven EONS (see Table 3.) There was no statistically significant association between Human Immunodeficiency virus exposure (12/27) and Gram-negative EONS p value = 0.101.

Table 4 shows the continuous variables of newborns with culture-proven EONS and those without EONS. The length of hospital stay was statistically significantly longer in newborns with culture-proven EONS. There were no statistically significant differences between the other continuous variables.

Table 4: Continuous variables of newborns with culture-proven EONS and those without EONS born and admitted into the neonatal unit, 1 January 2013 to 31 December 2018.

Variable	Culture-proven EONS	Total n	No EONS	Total n	*p*-value *
Birth weight (grams)	1975 (IQR 1803)	100	2010 (IQR 1640)	7619	0.989
Gestational age (weeks)	33.38 (SD 5.039)	100	34.23 (SD 4.739)	7612	0.075
Maternal age (years)	27.76 (SD 6.116)	93	28.75 (SD 6.264)	7262	0.634
Length of stay (days)	11 (IQR 16)	89	7 (IQR 17)	6602	<0.001

** Mann Whitney U used for Birth weight and Length of stay and Mean difference used for Gestational age and Maternal age*

Mortality within the culture-proven EONS group

Mortality was significantly higher in Gram-negative EONS 12/27 (44.4%) as compared with Gram-positive EONS 9/73 (12.33%) $p < 0.001$.

Klebsiella pneumoniae and *Escherichia coli* (*E. coli*) EONS were associated with significantly higher mortality. *Klebsiella pneumoniae* mortality was 5/10 $p < 0.001$ and *E. coli* mortality was 3/9 $p = 0.041$.

4. Discussion

This retrospective study reviewed the epidemiology of culture-proven EONS in a tertiary hospital in Johannesburg. We found that Gram-positive EONS accounts for the majority of EONS in this unit. Gram-negative culture-proven EONS was strongly associated with mortality, in our unit.

Over the study period, the majority of EONS was caused by Gram-positive organisms (73%). Group B Streptococcus was the most common Gram-positive isolate. This result is comparable with a study in Soweto, South Africa, between August 2013 and September 2014, where 71% of pathogens associated with EONS were Gram-positive organisms.(4) Current empiric antibiotic therapy using ampicillin, in our study site, to cover for Gram-positive sepsis remains a rational choice.

Group B Streptococcus was the most common organism, over the study period (n=37/100, 37%). This correlates with studies elsewhere in South Africa highlighting the high burden of GBS infection.(11, 12) In a study in Soweto, South Africa, the incidence of invasive early-onset GBS disease (< 7 days) was 1.41 per 1 000 live births.(12) CMJAH uses risk-based screening rather than a culture-based screening approach to prevent vertical transmission of GBS. The fluctuating per annum incidence of GBS, in

the CMJAH neonatal unit, may be associated with the inadequacies in the risk-based screening approach used in the obstetrics unit and late presentation of mothers who may have benefited from intrapartum antibiotic prophylaxis. Understanding epidemiology provides an approach to reduce neonatal deaths. Developing a vaccine, as an alternative preventative strategy, to immunize pregnant women from GBS prevalent serotypes could reduce the incidence of neonatal invasive GBS disease.(11, 12)

In our study, 34 newborns were excluded as they cultured a contaminant on blood culture. Contamination of blood culture arises when organisms not present in the bloodstream, mostly present as skin flora, are grown in culture. The false-positive cultures result in clinical uncertainty, increased antibiotic usage, increased duration of stay and increased health care costs.(13) Rates of contamination of blood cultures in the neonatal population vary considerably between neonatal units from as little as 0.6% to over 6%.(13) Neonatal units need to design procedures to reduce the rates of blood culture contamination. Methods to reduce contamination include sterile collection bundles of care. This includes strict hand hygiene, use of chlorhexidine cleaning solutions, sterile gloves, sterile blood culture collection packs, dedicated phlebotomy teams, and ongoing education and training programmes.(14) Continued surveillance and epidemiological studies are also crucial to monitor rates of false-positive results and to introduce quality improvement cycles.

In some studies of EONS, CoNS is always considered a contaminant and excluded from analysis, as it is difficult to decide whether CoNS is a clinically significant isolate or not. (4, 15) In this review, we excluded 86 newborns who cultured CoNS. In a review in the CMJAH neonatal unit in 2009 to 2010, CoNS was the most common overall isolate in EONS and late-onset neonatal sepsis (n=47/246, 19.1%).(6) In the CMJAH neonatal unit in 2012, CoNS accounted for 30.8% of EONS (n=12/39).(16) The clinical significance of CoNS remains difficult to interpret because they are found abundantly as natural skin commensals.(15) CoNS are generally associated with low mortality. However, CoNS strains are associated with an increasing pattern of antibiotic resistance especially to B-lactam antibiotics.(17, 18) The use of empiric antibiotic in neonates and the use of

maternal antibiotic prophylaxis may be driving the development of bacterial resistance to commonly used antibiotics within neonatal units. In light of emerging antibiotic resistance patterns of CoNS, it is crucial to find means to accurately identify CoNS as true pathogens from contaminants.

During the period 2013 to 2016, there were no documented sporadic *Listeria monocytogenes* infections. From 1 January 2017 to 31 December 2018, there were eleven *Listeria* cases, which corresponds with the *Listeria* epidemic in South Africa from mid-June 2017 to July 2018.(19) The mortality associated with *Listeria* cases at the CMJAH unit was 18% (n=2/11). In an audit from Tygerberg Hospital, Cape Town, from 1 January 2017 to 31 January 2018, the mortality in confirmed *Listeria* cases was 25% (n=3/12).(20) Regular auditing of EONS within neonatal units assists with surveillance and early warning of potential outbreaks and ensures directed antibiotic treatment.

In our study, chorioamnionitis was not strongly associated with EONS. This may be, in part, as a result of the narrow diagnostic criteria used to define chorioamnionitis and inadequate identification of mothers with clinical features of chorioamnionitis. Interestingly, studies have shown that in premature newborns the presence of chorioamnionitis has been associated with reductions in respiratory distress syndrome, chronic lung disease and mortality. (21)

In our study, 64.9% (4987/7681) of the deliveries were via CS, which is much greater than that proposed by the International Healthcare Community of between 10-15%.(22) CMJAH is a tertiary referral centre for high risk obstetrics cases, likely contributing to the high rate of CS. We found vaginal delivery to be strongly associated with EONS. This association may be as a result of vaginal colonization with pathogenic bacteria resulting in EONS.

Gram-negative EONS was strongly associated with higher mortality which is in keeping with the audit in the same unit from 2009 to 2010.(6) *E. coli* was the second most common Gram-negative organism (n=9/100, 9%) and this is in keeping with a study in

Soweto, South Africa, where *E. coli* accounted for 8% of culture-proven EONS (n=8/99).(4) In the developed world, *E. coli* is estimated to be the second leading cause of EONS.(23) *Klebsiella pneumoniae* was the predominant Gram-negative organism in our study (n=10/100, 10%), however *Klebsiella pneumonia* was less frequently isolated in the study in Soweto (1%).(4) Variability in incidence of Gram-negative sepsis may be, in part, affected by the use of maternal antibiotic prophylaxis.(23) Gentamicin seems an appropriate antibiotic, in our study site, to be used synergistically with ampicillin to cover for Gram-negative organisms.

Culture-proven EONS was associated with higher mortality, in our study. This result is consistent with the 2016 PPIP data which lists sepsis as one of the leading causes of neonatal deaths in South Africa.(3) This result is also consistent with the global estimates of severe infection being a direct cause of neonatal deaths.(2) In order to target the sustainable development goal for NMR, efforts need to be intensified in LMICs to address preventable early neonatal deaths. Improved quality and timeous data collection remains essential to strategize how to reduce neonatal deaths related to EONS.(2)

5. Limitations

This was a retrospective review of an existing database. There was no control over how newborns identified to have clinical sepsis had bacterial blood cultures drawn in terms of sterility and volume of blood drawn.

The lack of a consensus definition for EONS results in difficulties comparing existing research.

CMJAH is a tertiary level academic hospital dealing with both high-risk pregnancies and deliveries and this may create limitations regarding the generalizability of this this study to the larger South African neonatal population.

6. Conclusion

Epidemiological review of culture-proven EONS, within neonatal units, remains an important practice. Gram-positive EONS still predominates in the CMJAH neonatal unit, with GBS remaining an important cause of EONS. Mortality is strongly associated with Gram-negative EONS within the unit, specifically *Klebsiella pneumoniae* and *E. coli*. By regular auditing we are able to improve our understanding of organisms associated with EONS, implement strategies designed to reduce the NMR, and compare current research meaningfully.

7. References

1. World Health Organization. (2006). Neonatal and perinatal mortality: country, regional and global estimates. *World Health Organization*. Available at: https://apps.who.int/iris/handle/10665/43444. Accessed on: 31/07/2019

2. Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: When? Where? Why? *The Lancet*. 2005;365(9462):891-900. https://doi.org/10.1016/S0140-6736(05)71048-5

3. Rhoda N, Velaphi S, Gebhardt GS, Kauchali S, Barron P. Reducing neonatal deaths in South Africa: Progress and challenges. *South African Medical Journal*. 2018;108(3 Suppl 1):S9-S16. https://doi.org/10.7196/SAMJ.2018.v108i3.12804

4. Velaphi SC, Westercamp M, Moleleki M, Pondo T, Dangor Z, Wolter N, et al. Surveillance for incidence and etiology of early-onset neonatal sepsis in Soweto, South Africa. *PLoS One*. 2019;14(4):1-18. https://doi.org/10.1371/journal.pone.0214077

5. Wynn JL, Wong HR, Shanley TP, Bizzarro MJ, Saiman L, Polin RA. Time for a neonatal-specific consensus definition for sepsis. *Pediatric Critical Care Medicine*. 2014;15(6):523-528. https://doi.org/10.1097/PCC.0000000000000157

6. Ballot DE, Nana T, Sriruttan C, Cooper PA. Bacterial bloodstream infections in neonates in a developing country. *ISRN Pediatrics*. 2012:1-6. https://doi.org/10.5402/2012/508512

7. Sankar MJ, Natarajan CK, Das RR, Agarwal R, Chandrasekaran A, Paul VK. When do newborns die? A systematic review of timing of overall and cause-specific neonatal deaths in developing countries. *Journal of Perinatology.* 2016;36:S1-S11. https://doi.org/10.1038/jp.2016.27

8. Seale AC, Mwaniki M, Newton CRJC, Berkley JA. Maternal and early onset neonatal bacterial sepsis: burden and strategies for prevention in sub-Saharan Africa. *The Lancet Infectious Diseases*. 2009;9(7):428-438. https://doi.org/10.1016/S1473-3099(09)70172-0

9. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of Biomedical Informatics*. 2009;42(2):377-381. https://doi.org/10.1016/j.jbi.2008.08.010

10. Papile L-A, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. *The Journal of Pediatrics*. 1978;92(4):529-534. https://doi.org/10.1016/s0022-3476(78)80282-0

11. Dangor Z, Lala SG, Cutland CL, Koen A, Jose L, Nakwa F, et al. Burden of invasive group B Streptococcus disease and early neurological sequelae in South African infants. *PLoS One*. 2015;10(4):1-13. https://doi.org/10.1371/journal.pone.0123014

12. Dangor Z, Cutland CL, Izu A, Kwatra G, Trenor S, Lala SG, et al. Temporal changes in invasive group B Streptococcus serotypes: Implications for vaccine development. *PLoS One*. 2016;11(12):1-12. https://doi.org/10.1371/journal.pone.016910

13. Hall KK, Lyman JA. Updated review of blood culture contamination. *Clinical Microbiology Reviews*. 2006;19(4):788-802. https://doi.org/10.1128/CMR.00062-05

14. Hamilton LF, Gillett HE, Smith-Collins A, Davis JW. A sterile collection bundle intervention reduces the recovery of bacteria from neonatal blood culture. *Biomedicine Hub*. 2018;3(1):1-7. https://doi.org/10.1159/000486703

15. Marchant EA, Boyce GK, Sadarangani M, Lavoie PM. Neonatal Sepsis due to Coagulase-Negative Staphylococci. *Journal of Immunology Research*. 2013:1-10. https://doi.org/10.1155/2013/58607

16. Lebea MM, Davies V. Evaluation of culture-proven neonatal sepsis at a tertiary care hospital in South Africa. *South African Journal of Child Health*. 2017;11(4):170-173. https://doi.org/10.7196/SAJCH.2017.v11i4.1310

17. Nazir A. Neonatal sepsis due to coagulase negative Staphylococci: a study from Kashmir valley, India. *International Journal of Contemporary Pediatric*s. 2019;6(2):650-655. http://dx.doi.org/10.18203/2349-3291.ijcp20190705

18. Hira V, Sluijter M, Goessens WHF, Ott A, de Groot R, Hermans PWM, et al. Coagulase-negative staphylococcal skin carriage among neonatal intensive care unit personnel: from population to infection. *Journal of Clinical Microbiolog*y. 2010;48(11):3876-3881. https://doi.org/10.1128/JCM.00967-10

19. Smith AM, Tau NP, Smouse SL, Allam M, Ismail A, Ramalwa NR, et al. Outbreak of Listeria monocytogenes in South Africa, 2017–2018: laboratory activities and experiences associated with whole-genome sequencing analysis of isolates.

Foodborne Pathogens and Disease. 2019;16(7):524-530. https://doi.org/10.1089/fpd.2018.2586

20. Dramowski A, Lloyd LG, Bekker A, Holgate S, Aucamp M, Reddy K, et al. Neonatal listeriosis during a countrywide epidemic in South Africa: A tertiary hospital's experience. *South African Medical Journal*. 2018;108(10):818-827. https://doi.org/10.7196/SAMJ.2018.v108i10.13207

21. Rayond SL, Rincon JC, Wynn JL, Moldawer LL, Larson SD. Impact of Early-Life Exposures to Infections, Antibiotics, and Vaccines on Perinatal and Long-term Health and Disease. *Frontiers in Immunology.*2017;8:729. https://doi.org/10.3389/fimmu.2017.00729

22. World Health Organisation. (2015). WHO Statement on Caesarean Section Rates. *World Health Organization*. Available at: https://www.who.int/reproductivehealth/publications/maternal_perinatal_health/cs-statement/en/. Accessed on:13/01/2020

23. Simonsen KA, Anderson-Berry AL, Delair SF, Davies HD. Early-onset neonatal sepsis. C*linical Microbiology Reviews*. 2014;27(1):21-47. https://doi.org/10.1128/CMR.00031-13

APPENDICES

Appendix A: Instructions for authors

SAJID | SOUTHERN AFRICAN JOURNAL OF INFECTIOUS DISEASES

Overview

The author guidelines include information about the types of articles received for publication and preparing a manuscript for submission. Other relevant information about the journal's policies and the reviewing process can be found under the about section.
The **compulsory cover letter** forms part of a submission and must be submitted together with all the required forms. All forms need to be completed in English.

Original Research Article

An original article provides an overview of innovative research in a particular field within or related to the focus and scope of the journal, presented according to a clear and well-structured format.

Word limit	3500 words (excluding the structured abstract and references)
Structured abstract	250 words to include a Background, Methods, Results and Conclusion
References	50 or less
Tables/Figures	no more than 7 Tables/Figure
Ethical statement	should be included in the manuscript
Compulsory supplementary file	ethical clearance letter/certificate

Original Research Article full structure

Title: The article's full title should contain a maximum of 95 characters (including spaces).

Abstract: The abstract, written in English, should be no longer than 250 words and must be written in the past tense. The abstract should give a succinct account of the objectives, methods, results and significance of the matter. The structured abstract for an Original Research article should consist of four paragraphs labelled Background, Methods, Results and Conclusion.

- Background: Summarise the social value (importance, relevance) and scientific value (knowledge gap) that your study addresses.
- Methods: Clearly express the basic design of the study, and name or briefly describe the methods used without going into excessive detail.
- Results: State the main findings.
- Conclusion: State your conclusion and any key implications or recommendations.
  Do not cite references and do not use abbreviations excessively in the abstract.

Introduction: The introduction must contain your argument for the social and scientific value of the study, as well as the aim and objectives:

- Social value: The first part of the introduction should make a clear and logical argument for the importance or relevance of the study. Your argument should be supported by use of evidence from the literature.
- Scientific value: The second part of the introduction should make a clear and logical argument for the originality of the study. This should include a summary of what is already known about the research question or specific topic, and should clarify the knowledge gap that this study will address. Your argument should be supported by use of evidence from the literature.
- Conceptual framework: In some research articles it will also be important to describe the underlying theoretical basis for the research and how these theories are linked together in a conceptual framework. The theoretical evidence used to construct the conceptual framework should be referenced from the literature.
- Aim and objectives: The introduction should conclude with a clear summary of the aim and objectives of this study.

Research methods and design: This must address the following:

- Study design: An outline of the type of study design.

- Setting: A description of the setting for the study; for example, the type of community from which the participants came or the nature of the health system and services in which the study is conducted.
- Study population and sampling strategy: Describe the study population and any inclusion or exclusion criteria. Describe the intended sample size and your sample size calculation or justification. Describe the sampling strategy used. Describe in practical terms how this was implemented.
- Intervention (if appropriate): If there were intervention and comparison groups, describe the intervention in detail and what happened to the comparison groups.
- Data collection: Define the data collection tools that were used and their validity. Describe in practical terms how data were collected and any key issues involved, e.g. language barriers.
- Data analysis: Describe how data were captured, checked and cleaned. Describe the analysis process, for example, the statistical tests used or steps followed in qualitative data analysis.
- Ethical considerations: Approval must have been obtained for all studies from the author's institution or other relevant ethics committee and the institution's name and permit numbers should be stated here.

Results: Present the results of your study in a logical sequence that addresses the aim and objectives of your study. Use tables and figures as required to present your findings. Use quotations as required to establish your interpretation of qualitative data. All units should conform to the **SI convention** and be abbreviated accordingly. Metric units and their international symbols are used throughout, as is the decimal point (not the decimal comma).

Discussion: The discussion section should address the following four elements:

- Key findings: Summarise the key findings without reiterating details of the results.
- Discussion of key findings: Explain how the key findings relate to previous research or to existing knowledge, practice or policy.
- Strengths and limitations: Describe the strengths and limitations of your methods and what the reader should take into account when interpreting your results.

- Implications or recommendations: State the implications of your study or recommendations for future research (questions that remain unanswered), policy or practice. Make sure that the recommendations flow directly from your findings.

  Conclusion: Provide a brief conclusion that summarises the results and their meaning or significance in relation to each objective of the study.

  Acknowledgements: Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution. Authors are responsible for ensuring that anyone named in the Acknowledgments agrees to be named. Refer to the acknowledgement structure guide on our *Formatting Requirements* page.

  Also provide the following, each under their own heading:

- Competing interests: This section should list specific competing interests associated with any of the authors. If authors declare that no competing interests exist, the article will include a statement to this effect: *The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.* Read our **policy on competing interests**.
- Author contributions: All authors must meet the criteria for authorship as outlined in the **authorship** policy and **author contribution** statement policies.
- Funding: Provide information on funding if relevant
- Data availability: All research articles are encouraged to have a data availability statement.
- Disclaimer: A statement that the views expressed in the submitted article are his or her own and not an official position of the institution or funder.

  References: Authors should provide direct references to original research sources whenever possible. References should not be used by authors, editors, or peer reviewers to promote self-interests. Refer to the journal referencing style downloadable on our *Formatting Requirements* page.

Appendix B: Approved Research Protocol

EPIDEMIOLOGY OF CULTURE PROVEN EARLY-ONSET NEONATAL SEPSIS IN A TERTIARY HOSPITAL IN JOHANNESBURG

Tracey Lauren Turner
2067280
MBCHB

Supervisors
Professor Daynia Ballot
MBBCh: FCPaed SA; PhD

Dr Robin Saggers
MBBCh, FCPaed, MMed (Paed)

BACKGROUND

Worldwide, each year, an estimated 4 million babies die in the neonatal period and of these babies 75% will die within the first week of life with the highest risk of death on the first day of life (2). Ninety nine percent of neonatal deaths arise in low and middle income countries and half of these neonates will die at home without reaching health care facilities and without being documented in any formal registration system (2) (6).

The majority, 81%, of neonatal deaths in South Africa occur within the first week of life (3). Data from the 2016 STATS SA report, Perinatal deaths in South Africa, which collates data from the Department of Home Affairs (DHA), captured 6722 early neonatal deaths (0-7days) in South Africa for the year 2016 with 69.8% of these deaths occurring within the first three days of life (25). In this statistical release, Gauteng province contributed to the largest proportion of early neonatal deaths in the country at 28% of all early neonatal deaths (25). The neonatal mortality rate (NMR) is the number of neonatal deaths (deaths in the first 28 days of life) per 1000 live births. The NMR is a key indicator for newborn care and directly reflects prenatal, intrapartum and neonatal care. The South African Demographic Health Survey reported a NMR of 21-29 per 1000 live births for the 5 years preceding the 2016 survey. Rapid mortality surveillance reports, produced by the Medical Research Council, indicate that based on statistics, from Statistics SA, between 2012 and 2015 NMR has remained the same at 11-12 per 1000 live births in South Africa (26). According to the 2016 Perinatal Problem Identification Programme data, the leading causes of neonatal deaths in South Africa are: complications of prematurity, intrapartum-related events and infections (3). Both these NMR rates as well as the data collected from DHA seem discordant with the World Health Organization estimation of NMR in sub-Saharan Africa of 44 per 1000 live births (2). The discordance in NMR and limitations in establishing why and how newborns die may be attributed to: lack of reliable birth and death registration data (3), inadequate data capturing of neonatal specific causes of death, deaths occurring outside healthcare facilities

(3), misclassification of neonatal deaths as stillbirths (27), allocation of a death to an individual cause where multiple factors may play a role in a death as well as lack of diagnostic tests which have a high sensitivity and specificity for neonatal sepsis (4). In order to be in line with meeting the sustainable development goal (SDG) 3 and target a NMR of 12 per 1000 live births by 2030, South Africa must have timely accurate data and the country's efforts to reduce the NMR need to be intensified (3, 28).

Neonatal infections continue to cause significant mortality and morbidity in neonatal units, across the globe. Severe infections contribute to the majority, 36%, of all causes of neonatal deaths (2). Periodically reviewing sepsis as a direct cause of neonatal mortality is of great public health importance in order to drive better service delivery, guide programmes designed to reduce NMR and in order to reduce the emergence of antimicrobial resistance.

Early–onset neonatal sepsis (EONS) occurs within 72 hours of life and is considered to be as a result of bacterial organism transfer intra, peri or post-partum via the maternal genital tract (17). Organisms associated with EONS colonise the maternal recto and genitourinary tract and result in: amniotic fluid, placental, cervical or vaginal contamination (24). Risk factors for EONS can be organized into maternal and neonatal factors. Neonatal factors associated with EONS include: prematurity, low birth weight, congenital anomalies, complications of assisted deliveries, low Apgar scores, innate neonatal immunological deficiencies, impaired skin barrier function, invasive skin breaching procedures and invasive ventilation (3, 24, 29). Maternal risk factors for EONS include: ingestion of contaminated foods, procedures during pregnancy (cerclage and amniocentesis), prolonged rupture of membranes, maternal fever, colonization with group B *streptococcus,* maternal immunity, difficulties at delivery (birth asphyxia or obstructed labour), chorioamnionitis and meconium stained amniotic fluid (7, 24).

Definitions of neonatal sepsis are variable and there is lack of a neonatal–specific consensus definition for sepsis (5). Having a positive culture is often considered to be the “gold standard” in the definition of infection, however culture-negative or clinical sepsis is a well known neonatal entity (5). The entity of culture-negative neonatal sepsis may represent nearly 60% of neonates presenting with clinical sepsis (30) and this contributes to the low reporting of neonatal sepsis in neonatal units around the world.

Three retrospective research reports reviewed culture proven sepsis at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH). A study from June 2002 to July 2003 reviewed the epidemiology of neonatal sepsis. In this study there was an EONS incidence of 0.4 per 1000 live births (n=5) (31). A second study between June 2009 to June 2010 reviewed sensitivity patterns of all neonatal bacterial blood stream infections (11) and the incidence of EONS was 0.93 per 1000 live births (n=16). The third study from January 2012 to December 2012 evaluated culture-proven neonatal sepsis. In this study there was an incidence of EONS of 1.7 per 100 admissions (n=33) (16). In a study at Chris Hani Baragwanath Academic Hospital, in Soweto, from August 2013 to September 2014 the incidence of culture-confirmed EONS was 3.2 per 1000 live births (4). Periodic review of pathogen associated causes of EONS is an essential part of effective infection control strategies and is essential in guiding the use of empiric antibiotic therapy (11).

Organisms causing Early Onset Neonatal Sepsis

Organisms causing EONS are broadly classified according to their initial microbiological gram stain as gram-positive or gram-negative. Identification of organisms according to their gram stains has important implications in guiding physicians with choosing an empiric antibiotic regimen.

The most common organisms causing EONS in the CMJAH neonatal unit in 2009 were coagulase-negative *staphylococci* (CoNS) (n=12/39) and CoNS were

the second commonest organism causing EONS in 2009/2010 (n=4/16) and the situation is similar in other neonatal intensive care units worldwide (17, 18). CoNS are gram-positive organisms and common inhabitants of the skin and mucous membranes and transmission primarily occurs via horizontal transmission but vertical transmission also occurs (32). Poor aseptic technique in collecting blood cultures may be a cause of blood culture contamination and this may result in over treating neonates and lead to the development of antibiotic resistance. The clinical significance of CoNS is difficult to interpret because they are found abundantly as a natural skin commensal (11) (32) and are generally associated with low mortality. However CoNS strains are associated with an increasing pattern of antibiotic resistance especially to B-lactam antibiotics (17) (18) and rates of methicillin resistance can be up to 80% - 86% (11). Distinguishing true infection from blood culture contamination by means of collecting multiple blood cultures prior to initiating antibiotics, in the critically ill neonate, is not possible. Postponing the initiation of antibiotics whilst awaiting bacterial confirmation of culture specimens, in neonates, is also not practical. The non-specific laboratory and clinical signs of neonatal sepsis along with the rapid progression of neonatal sepsis makes it difficult to interpret blood specimens that have cultured CoNS and to decide whether to treat or not to treat. Prompt diagnosis and early antibiotic therapy in CoNS sepsis remains crucial in neonates because of the significant morbidity, prolonged hospital stays and considerable costs to neonatal units (17, 29). It has become crucial to identify CoNS as true pathogens from contaminants in light of the emerging antibiotic resistance patterns of CoNS organisms.

Group B *streptococcus* (GBS) also known as *Streptococcus agalactiae* accounted for only 2/39 cases of EONS in 2012 but in 2009/2010 it was the predominant pathogen causing EONS (n=7/17) (11, 16). Variability in isolating GBS has been noted in other neonatal units. A study over a twelve month period, at three secondary-tertiary care public hospitals in Johannesburg, highlighted the high burden of invasive GBS disease as well as the developmental problems

associated with surviving GBS meningitis in South Africa (10). An incidence of early onset disease, within seven days of life, was 1.37 per 1000 live births in this study (n=43) (10). This incidence is in contrast to the situation in the United States where there has been a notable decline by at least 80% (24) in the incidence of early-onset GBS and this could be attributed to the use of prenatal screening and intra - partum antibiotic prophylaxis (IAP) whereas in the south African context only a quarter of women eligible for IAP receive this treatment timeously (10).

Staphylococcus aureus and more concerning methicillin resistant *Staphylococcus aureus* (MRSA) is a cause of gram-positive EONS. *Staphylococcus aureus* infection contributed to (n=3/39) of culture proven EONS in 2012 (16). Similar to CoNS, *Staphylococcus aureus* is a ubiquitous human inhabitant and colonization predominates the anterior nares. MRSA is associated with significant mortality and morbidity including adverse long term neonatal outcomes resulting in substantial disease burden (33). The emergence of vancomycin resistant *S. aureus* poses huge challenges for anti-microbial treatment options in neonates.

Listeria Monocytogenes (LM) is a Gram-positive ubiquitous food-borne pathogen that causes the disease named listeriosis. *Listeria* is associated with severe and invasive infections in immune-compromised hosts including pregnant women and newborns. Transmission from pregnant mother, transplacentally or during and after delivery via contaminated amniotic fluid, results in EONS and these neonates present with severe disease and experience high mortality. LM is associated with sporadic and epidemic human infections mostly from high-income countries and seen less frequently in developing countries (20). *Listeria* was not isolated as a cause for EONS in the CMJAH unit in 2009/2010 nor in 2012 (16). In South Africa, the world's largest documented outbreak of listeriosis was noted from mid June 2017 - July 2018 with a total of 1060 cases reported from January 2017 to July 2018 with most of the cases, 59% arising from

Gauteng. The source of this outbreak was related to a ready-to-eat processed meat production facility, *Enterprise Foods*, in Polokwane, South Africa (19). This outbreak highlights the importance of surveillance in units. According to the National institute for communicable diseases (NICD) neonatal LM bacteraemia and meningitis is treated according to the standard treatment guidelines and essential medicines list for South Africa with intravenous ampicillin with or without gentamicin, at the discretion of the attending physician.

Extended-spectrum beta-lactamase (ESBL) - producing *Enterobacteriaceae* are gram-negative bacteria of major clinical concern. ESBL-producing *Klebsiella pneumoniae* accounted for 1/16 and 9/39 organisms causing EONS in the CMJAH unit in 2009/2010 and 2012 respectively (11, 16). ESBL organisms are pathogens associated with outbreaks in neonatal units, worldwide, and their presence carries with it tremendous clinical significance (34). ESBL organisms colonize the gastro intestinal tract of patients as well as contaminating the environment (mattresses, incubators, glove boxes, scales and ultrasound gels) within neonatal units acting as reservoirs for future continued transmissions via person-to-person spread. ESBL- producing bacteria produce a challenge to neonatal units in terms of antibiotic treatment options, as resistance patterns to empiric antibiotics are high, as well as the high rates of neonatal mortality associated with resistant gram-negative organisms (11, 35). The treatment choice for serious infections due to ESBL-producing organisms are the carbapenems, however there has been an emergence in quinolone and carbapenem-resistant isolates and this already has created significant therapeutic challenges (34).

Acinetobacter baumannii (A. *baumannii*), a gram-negative aerobic cocco-bacilli, is also emerging as a cause of sporadic EONS in newborns and is also implicated in outbreaks in neonatal units as a result of their ability to survive within hospital environs for prolonged periods. A *baumannii* EONS accounted for 7/39 isolated in the CMJAH unit in 2012. A. *baumannii* have the ability to develop

resistance to various classes of antimicrobials by producing B-lactamases resulting in both multi and extremely drug resistant pathogens. A. *baumannii* is also associated with: longer hospitalization, risks for further adverse advents, decreased family bonding, high costs to neonatal units and higher overall neonatal mortality (36).

Escherichia coli (E.coli) are gram-negative bacteria which frequently colonize the maternal vaginal canal. Neonates acquire the infection at or just after delivery. *E. Coli* are estimated as the second leading cause of EONS in the developed world and are frequently associated with: severe infections, meningitis, high mortality rates, high rates of adverse complications and long term disabilities in neonates (23). In contrast in CMJAH, *E. Coli* contributed to no EONS in 2009/2010 and 2/39 cases of EONS in 2012. The incidence of *E. Coli* EONS may be in part affected by use of maternal antibiotic prophylaxis (24) and or the increasing survival of very low birth weight infants. *E. Coli* is the most frequent cause of EONS when very low birth weight neonates are considered alone (24). There is also an emergence of ampicillin-resistant *E. coli* isolates in neonatal units which impacts empiric antibiotic choice (37).

Incidence and pathogens associated with EONS differ widely between countries. Data suggest that EONS accounts for a high burden of disease especially in Sub Saharan Africa, however incidence and aetiologies of infections are largely unknown and remain elusive in low-middle income countries (4, 7). In order to continue to improve our understanding of EONS uniformity in definitions of neonatal sepsis need to be addressed. Future epidemiological large population based studies addressing pathogens associated with EONS and identifying risk factors associated with EONS need to be conducted in order to reduce neonatal deaths (7). Neonatal units are obliged to periodically review EONS epidemiological patterns in order to: guide empiric antibiotic prescriptions, improve neonatal mortality, guide policy makers to devise policies to address the quality of maternal and newborn care (6), aid in development of vaccines against

prevalent pathogens (7), meet SDG targets, reduce resistance to antibiotics as well as advocate for stricter infection control measures within neonatal units.

The aim of this study is to review the epidemiology of culture proven EONS.

OBJECTIVES

- To describe the clinical and demographic characteristics of the study sample.
- To compare the prevalence and causative organisms of culture-confirmed EONS (<72hours), per annum, over the study period.
- To describe risk factors and in hospital survival for EONS by comparing neonates with and without EONS.

PATIENTS AND METHODS

Study site

The neonatal unit at CMJAH is a tertiary academic neonatal unit in Johannesburg, South Africa. The 99 bedded neonatal unit is comprised of 5 clinical areas: a 14 bedded combined medical and surgical intensive care unit which serves both paediatric and neonatal patients, a 10 bedded transitional unit, a 35 bedded high care unit, a 30 bedded low care neonatal ward and a 10 bedded kangaroo mother care unit. The unit services both inborn babies and ill outborn neonates who are transferred in from peripheral drainage clinics and hospitals. CMJAH neonatal unit services about 15000 deliveries per annum – both in hospital and at satellite midwifery obstetric units.

As a standard of care, all neonates who are assessed by a physician to have a diagnosis of a suspected serious bacterial infection are admitted to the CMJAH unit and get a routine laboratory work up for sepsis. This work up for sepsis includes; a full blood count with a differential count, an aseptically collected blood culture and a C- reactive protein. Empiric intravenous antibiotics are initiated after cultures have been taken. Current empiric antibiotic choice in the CMJAH

neonatal unit includes the use of ampicillin to cover potential gram-positive organisms and an aminoglycoside, gentamicin, to cover potential gram-negative organisms.

Study sample

All newborns admitted to the CMJAH neonatal unit between 1 January 2013 and 31 December 2018 are eligible for inclusion in the study.

Inclusion criteria: neonates who were born in CMJAH and admitted to the CMJAH neonatal unit.

Exclusion criteria: neonates with late onset neonatal sepsis (>72hours).

Data collection

Data for this retrospective descriptive study will be collected from two main sources.

The first source is an existing neonatal computer database. There is ongoing data collection for the purpose of clinical auditing and quality improvement. Information is collected from all neonates admitted to the neonatal unit, upon discharge or death. Data is collected weekly by attending medical staff on data collection sheets, which are verified against hospital records. Data is entered onto the computer database and is checked again. Data is managed using Research Electronic Data Capture (REDCAP) hosted by the University of the Witwatersrand (8). Relevant obstetric, demographic and clinical information will be collected for each subject (see data collection sheet and definitions below).

Definitions

Culture-confirmed EONS: isolation of a pathogenic bacterial organism in the blood stream of a neonate before 72 hours of life associated with clinical signs and/or laboratory features suggestive of sepsis.

This study will not be equipped to investigate culture-negative EONS.

Cultures containing growth of bacteria namely: *Bacillus* species, *Corynebacteria* species, CoNS, *Micrococcus* species and *Streptococcus viridans* will be excluded from the study.

In the case of cultures growing more than one organism, results will be considered contaminants and will not included in the analysis.

A neonate will be considered exposed to human immunodeficiency virus (HIV) if born to a mother who was diagnosed antenatally or at delivery with HIV-1 or HIV-2.

APGAR score at 5 and 10 minutes will be documented as a proxy for neonatal encephalopathy.

Intraventricular haemorrhage (IVH) was documented based on Papile et al's cranial ultrasound grading as follows: Grade 3 - IVH with ventricular dilatation and Grade 4 -IVH extending into adjacent brain parenchyma (9) .

Sex will be based on appearance of external genitalia as male, female or unknown.

Mode of delivery will be classified as either vaginal for any vaginal delivery or caesarean section for any elective or emergency caesarean section.

Maternal antenatal care attendance will be considered as being received if a mother received any prenatal obstetrical care prior to the admission during which birth occurred and will be considered negative if she received no prenatal obstetrical care.

Clinical Data definitions are according to the Vermont Oxford Network (VON) 2019 manual of operations.

STATISTICAL ANALYSIS

All the demographic and laboratory data will be exported into Microsoft Excel 2010 for data cleaning and checking. The final data will be exported into IBM SPSS version 25 for analysis. The final sample will be described. Continuous variables will be described using mean and standard deviation, if normally distributed, and median and interquartile range if the distribution is skewed. Categorical variables will be described using frequencies and percentages. The sample will be divided into neonates with EONS and those without. In hospital survival, demographic, obstetric and clinical characteristics will be compared between these groups. The chi-square test will be used to compare categorical variables and unpaired t-tests will be used to compare continuous variables. A p-value of <0.05 will be considered as statistically significant. All variables with a p-value of <0.1 will be entered into a binary logistic regression to provide adjusted odds ratio for risk factors associated with EONS.

Sample size estimation

There are approximately 1500 inborn neonates admitted to the CMJAH neonatal unit per annum and about 10% of these neonates develop late onset sepsis and a further 10% do not have culture results. The total sample size is therefore estimates to be 6000 neonates. It is therefore estimated that there will be 240 neonates with EONS during this study period.

ETHICAL CONSIDERATIONS

Data will be de-identified and this information will be kept separately in a file only accessible to the investigator. De-identifying data pertaining to: personal names, birth date, date of death, hospital identification numbers and admission dates will preserve patient privacy, confidentiality and anonymity. The commencement of this study is provisional on approval from: the University of the Witwatersrand Human Research Ethics Committee, University of the Witwatersrand postgraduate committee and from the hospital Chief executive officer of CMJAH.

FUNDING

Funding for this study will all be from personal finances.

Stationery inter alia (printing costs, paper, pens) R300

Fibre costs R880 p/month

PROJECT MILESTONES/TIMING

RESEARCH REPORT TIMELINE	April - July 2019	October-19	Nov -19	Nov -19	Dec 2019 - Jan 2020	Dec -21
Literature Review						
Protocol Preparation						
Approval school-based Masters protocol assessments						
Approval Ethics committee (Online) Turn around time 28 days						
Data collection Research Block confirmed						
Processing of data						
Final Submission dates						

REFERENCES

1. Organization WH. Neonatal and perinatal mortality. France: World Health Organization; 2006 2006. Contract No.: WS 16 2006NE.
2. Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: When? Where? Why? The Lancet. 2005;365(9462):891-900.
3. Rhoda N, Velaphi S, Gebhardt GS, Kauchali S, Barron P. Reducing neonatal deaths in South Africa: Progress and challenges2018.
4. Velaphi SC, Westercamp M, Moleleki M, Pondo T, Dangor Z, Wolter N, et al. Surveillance for incidence and etiology of early-onset neonatal sepsis in Soweto, South Africa. PloS one. 2019;14(4):e0214077-e.
5. Wynn JL, Wong HR, Shanley TP, Bizzarro MJ, Saiman L, Polin RA. Time for a neonatal-specific consensus definition for sepsis. Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies. 2014;15(6):523-8.
6. Sankar MJ, Natarajan CK, Das RR, Agarwal R, Chandrasekaran A, Paul VK. When do newborns die? A systematic review of timing of overall and cause-specific neonatal deaths in developing countries. Journal of perinatology : official journal of the California Perinatal Association. 2016;36 Suppl 1(Suppl 1):S1-S11.

7. Seale AC, Mwaniki M, Newton CRJC, Berkley JA. Maternal and early onset neonatal bacterial sepsis: burden and strategies for prevention in sub-Saharan Africa. The Lancet Infectious diseases. 2009;9(7):428-38.
8. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009;42(2):377-81.
9. Papile L-A, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: A study of infants with birth weights less than 1,500 gm. The Journal of Pediatrics. 1978;92(4):529-34.
10. Dangor Z, Lala SG, Cutland CL, Koen A, Jose L, Nakwa F, et al. Burden of invasive group B Streptococcus disease and early neurological sequelae in South African infants. PLoS One. 2015;10(4):e0123014-e.
11. Ballot DE, Nana T, Sriruttan C, Cooper PA. Bacterial bloodstream infections in neonates in a developing country. ISRN Pediatr. 2012;2012:508512-.
12. Dangor Z, Cutland CL, Izu A, Kwatra G, Trenor S, Lala SG, et al. Temporal Changes in Invasive Group B Streptococcus Serotypes: Implications for Vaccine Development. PloS one. 2016;11(12):e0169101-e.
13. Hall KK, Lyman JA. Updated Review of Blood Culture Contamination. Clinical Microbiology Reviews. 2006;19(4):788-802.
14. Hamilton LF, Gillett HE, Smith-Collins A, Davis JW. A Sterile Collection Bundle Intervention Reduces the Recovery of Bacteria from Neonatal Blood Culture. Biomedicine Hub. 2018;3(1):1-7.
15. Bhat Y R, Lewis LES, K E V. Bacterial isolates of early-onset neonatal sepsis and their antibiotic susceptibility pattern between 1998 and 2004: an audit from a center in India. Italian journal of pediatrics. 2011;37:32-.
16. Lebea MM, Davies V. Evaluation Of Culture-Proven Neonatal Sepsis At A Tertiary Care Hospital In South Africa2017.
17. Nazir A. Neonatal sepsis due to coagulase negative Staphylococci: a study from Kashmir valley, India. 2019. 2019;6(2):6.
18. Hira V, Sluijter M, Goessens WHF, Ott A, de Groot R, Hermans PWM, et al. Coagulase-Negative Staphylococcal Skin Carriage among Neonatal Intensive Care Unit Personnel: from Population to Infection. Journal of Clinical Microbiology. 2010;48(11):3876-81.
19. Smith AM, Tau NP, Smouse SL, Allam M, Ismail A, Ramalwa NR, et al. Outbreak of Listeria monocytogenes in South Africa, 2017–2018: Laboratory Activities and Experiences Associated with Whole-Genome Sequencing Analysis of Isolates. Foodborne Pathog Dis. 2019;16(7):524-30.
20. Dramowski A, Lloyd LG, Bekker A, Holgate S, Aucamp M, Reddy K, et al. Neonatal listeriosis during a countrywide epidemic in South Africa: A tertiary hospital's experience2018.
21. Adatara P, Afaya A, Salia SM, Afaya RA, Kuug AK, Agbinku E, et al. Risk Factors for Neonatal Sepsis: A Retrospective Case-Control Study among Neonates Who Were Delivered by Caesarean Section at the Trauma and

Specialist Hospital, Winneba, Ghana. BioMed research international. 2018;2018:6153501-.
22. Badri T, Anurag T, Dhan Raj A, Kusum T, Asha P, Sudhir K, et al. Neonatal Sepsis as a Major Cause of Morbidity in a Tertiary Center in Kathmandu. Journal of Nepal Medical Association. 2013;52(192).
23. O'Rahelly MS, Aisling & Drew, R & Mccallion, Naomi Early Onset Neonatal E.Coli Sepsis. Irish medical journal. 2019;112.
24. Simonsen KA, Anderson-Berry AL, Delair SF, Davies HD. Early-Onset Neonatal Sepsis. Clinical Microbiology Reviews. 2014;27(1):21-47.
25. Maluleka R. Perinatal deaths in South Africa, 2016. In: Affairs NDoH, editor. Statistical release. South Africa2016. p. 44.
26. Dorrington RE BD, Laubscher R, Nannan N. Rapid mortality surveillance report 2016. Cape Town: South Africa, council CTSAMR; 2018.
27. Liu L, Kalter HD, Chu Y, Kazmi N, Koffi AK, Amouzou A, et al. Understanding Misclassification between Neonatal Deaths and Stillbirths: Empirical Evidence from Malawi. PLoS One. 2016;11(12):e0168743-e.
28. Grove J, Claeson M, Bryce J, Amouzou A, Ties B, Waiswa P, et al. Maternal, newborn, and child health and the Sustainable Development Goals--a call for sustained and improved measurement. Lancet. 2015;386.
29. Isaacs D. A ten year, multicentre study of coagulase negative staphylococcal infections in Australasian neonatal units. Archives of Disease in Childhood - Fetal and Neonatal Edition. 2003;88(2):F89.
30. Brocklehurst P, Farrell B, King A, Juszczak E, Darlow B, Haque K, et al. Treatment of neonatal sepsis with intravenous immune globulin. The New England Journal of Medicine. 2011;365(13):1201-11.
31. Motara F, Ballot DE, Perovic O. Epidemiology of Neonatal Sepsis at Johannesburg Hospital. Southern African Journal of Epidemiology and Infection. 2005;20(3):90-3.
32. Marchant EA, Boyce GK, Sadarangani M, Lavoie PM. Neonatal Sepsis due to Coagulase-Negative Staphylococci. Clinical and Developmental Immunology. 2013;2013:10.
33. Dong Y, Glaser K, Speer CP. New Threats from an Old Foe: Methicillin-Resistant Staphylococcus aureus Infections in Neonates. Neonatology. 2018;114(2):127-34.
34. Paterson DL, Bonomo RA. Extended-Spectrum β-Lactamases: a Clinical Update. Clinical Microbiology Reviews. 2005;18(4):657-86.
35. Khassawneh M, Khader Y, Abuqtaish N. Clinical features of neonatal sepsis caused by resistant Gram-negative bacteria. Pediatrics International. 2009;51(3):332-6.
36. Hsu J-F, Chu S-M, Lien R, Chiu C-H, Chiang M-C, Fu R-H, et al. Case-control analysis of endemic Acinetobacter baumannii bacteremia in the neonatal intensive care unit. American Journal of Infection Control. 2014;42(1):23-7.
37. Bergin SP, Thaden JT, Ericson JE, Cross H, Messina J, Clark RH, et al. Neonatal Escherichia coli Bloodstream Infections: Clinical Outcomes and Impact of Initial Antibiotic Therapy. Pediatr Infect Dis J. 2015;34(9):933-6.

EARCH REPORT DATA COLLECTION SHEET

DY NUMBER									
DE OF DELIVERY	VAGINAL	CAESAREAN							
CE OF BIRTH	INBORN	OUTBORN							
STATIONAL AGE eks	≤30	30-34	35-37	35-36	>37				
TH WEIGHT GRAMS	≤1000	1000-1499	1500-2499	≥2500					
	MALE	FEMALE							
EXPOSURE	EXPOSED	UNEXPOSED							
PHYXIA	APGAR <5 5MINS	APGAR >5							
TENATAL CARE	BOOKED	UNBOOKED							
GRADE 3/4	YES IVH	NO IVH							
OOD CULTURE	CONS	MSSA	MRSA	ENTEROCOCCUS FAECALIS	ENTEROCOCCUS FAECIUM	STREP AGALACTIAE	VIRIDANS STREPTOCOCCI	LISTERIA MONOCYTOGENI	
	KLEBSIELLA PNEUMONIAE	ESBL	E COLI	A BAUAMANNII					
	NEGATIVE CULTURE								
TIBIOTIC SENSITIVITY	PENICILLIN RESISTANT	AMINOGLYCOSIDE RESISTANT	MEROPENEM RESISTANT	CEFTAZIDIME RESISTANT					
C cells/mm3	<5 OR > 25	>5 OR < 25							
S cells/mm3	<150	≥150							
P > 10	> 10	< 10							
TCOME	ALIVE	DIED							

Appendix C: Plagiarism Declaration

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I, **Tracey Lauren Turner (Student number: 2067280**), am a student registered for the degree of **Master of Medicine (Paediatrics)** in the academic year **2021**.

I hereby declare the following:

- - I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- - I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- - I have followed the required conventions in referencing the thoughts and ideas of others.
- - I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- - I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: _____ _____ Date: 20/3/2021

Appendix D: Ethics Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Dr T Turner

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M191153

NAME: (Principal Investigator)	Dr T Turner
DEPARTMENT:	School of Clinical Medicine Department of Paediatrics and Child Health Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Epidemiology of culture proven early-onset neonatal sepsis in a tertiary hospital in Johannesburg
DATE CONSIDERED:	2019/11/29
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Professor D Ballot and Dr R Saggers
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2020/03/05

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Philip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. I **agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **November** and will therefore reports and re-certification will be due early in the month of **November** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

2020/03/05
Date

PLEASE QUOTE THE CLEARANCE CERTIFICATE NUMBER IN ALL ENQUIRIES

Appendix E: Turnitin Report

Turnitin Originality Report

- Processed on: 20-Mar-2021 8:10 AM SAST
- ID: 1537653260
- Word Count: 3279
- Submitted: 1

2067280:Submissable_paper_Turnitin_report_2021.docx By Tracey Turner

Similarity Index

14%

Similarity by Source

Internet Sources:
8%
Publications:
9%
Student Papers:
4%

2% match (publications)
Lydia Mudzikati, Angela Dramowski. "Neonatal septicaemia: prevalence and antimicrobial susceptibility patterns of common pathogens at Princess Marina Hospital, Botswana", Southern African Journal of Infectious Diseases, 2015
1% match (Internet from 07-Mar-2021)
https://bmcpediatr.biomedcentral.com/articles/10.1186/s12887-019-1709-y
1% match (Internet from 06-Aug-2017)
https://preview-mhnpjournal.biomedcentral.com/articles/10.1186/s40748-017-0048-y
1% match (publications)
N Rhoda, S Velaphi, G S Gebhardt, S Kauchali, P Barron. "Reducing neonatal deaths in South Africa: Progress and challenges", South African Medical Journal, 2018
1% match (publications)
"Posters", Clinical Microbiology and Infection, 2011
1% match (student papers from 21-Feb-2016)
Submitted to University College London on 2016-02-21
1% match (Internet from 30-Jul-2020)
https://www.frontiersin.org/articles/10.3389/fimmu.2017.00729/full
1% match (student papers from 22-Jan-2016)
Submitted to University of Witwatersrand on 2016-01-22
1% match (Internet from 19-Jul-2020)
http://www.sajch.org.za/index.php/SAJCH/article/view/709/593
1% match (Internet from 17-Mar-2021)
https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-020-05154-9
1% match (Internet from 15-May-2015)
https://www.wisconsinmedicalsociety.org/_WMS/publications/wmj/pdf/106/3/130.pdf
< 1% match (Internet from 09-Apr-2020)
https://obgyn.pericles-prod.literatumonline.com/doi/10.1002/ijgo.12505

< 1% match (Internet from 12-Nov-2020)
https://systematicreviewsjournal.biomedcentral.com/articles/10.1186/s13643-019-1207-1
< 1% match (Internet from 23-Feb-2021)
https://www.dovepress.com/prognostic-value-of-preoperative-absolute-lymphocyte-count-in-recurren-peer-reviewed-fulltext-article-OTT
< 1% match (publications)
Shouriyo Ghosh, Mandira Chakraborty, Sambit Samanta, Nilanjan Sinha et al. "Analysis of blood stream infections, antibiograms and clinical outcomes in haematological patients with febrile neutropenia: data from a tertiary care haematology institute in India", Annals of Hematology, 2020
< 1% match (student papers from 25-Feb-2019)
Submitted to University of Edinburgh on 2019-02-25
< 1% match (Internet from 28-Nov-2018)
https://link.springer.com/article/10.1186/s13104-018-3946-9
< 1% match (Internet from 11-Nov-2020)
https://pesquisa.bvsalud.org/gim/?lang=en%2Cau%3A%22Martins+Neto%2C+Viviana%22&q=au%3A%22Ballot%2C+D.%22
< 1% match (Internet from 27-May-2020)
https://sites.kowsarpub.com/hepatmon/articles/58442.html
< 1% match (Internet from 08-May-2020)
https://fjfsdata01prod.blob.core.windows.net/articles/files/482200/pubmed-zip/.versions/1/.package-entries/fpsyg-10-02736/fpsyg-10-02736.pdf?rscd=attachment%3B+filename%2A%3DUTF-8%27%27fpsyg-10-02736.pdf&se=2020-05-09T05%3A39%3A20Z&sig=Tv54%2Bk38VtOb9jn%2FHrbUa9XXOBnqe9bUPo9OWh%2F0Ru4%3D&sp=r&sr=b&sv=2015-12-11
< 1% match (publications)
Vicki A. Morrison. "Nosocomial septicemia in the cancer patient: The influence of central venous access devices, neutropenia, and type of malignancy", Medical and Pediatric Oncology, 1990
< 1% match (publications)
"Abstracts cont.", Clinical Microbiology and Infection, 2004
< 1% match (Internet from 28-Feb-2021)
https://www.hindawi.com/journals/ecam/2016/3587569/
< 1% match (Internet from 16-May-2016)
http://www.em-consulte.com/en/article/977447
< 1% match (Internet from 21-Jul-2020)
https://www.researchsquare.com/article/rs-36075/v1
< 1% match (Internet from 21-Feb-2021)
https://www.msdmanuals.com/professional/pediatrics/infections-in-neonates/neonatal-sepsis
< 1% match (publications)
Isabelle M. C. Ree, Suzanne F. Fustolo-Gunnink, Vincent Bekker, Karin J. Fijnvandraat, Sylke J. Steggerda, Enrico Lopriore. "Thrombocytopenia in neonatal sepsis: Incidence, severity and risk factors", PLOS ONE, 2017
< 1% match (publications)
Robert Pattinson, David Woods, David Greenfield, Sithembiso Velaphi. "Improving survival rates of newborn infants in South Africa", Reproductive Health, 2005
< 1% match (publications)
Nqoba Tsabedze, Jamie-Leigh Kinsey, Dineo Mpanya, Vanessa Mogashoa, Eric Klug, Pravin Manga. "The prevalence of depression, stress and anxiety symptoms in patients with chronic heart failure", Research Square, 2020