AN AUDIT OF CULTURE-PROVEN NEONATAL SEPSIS AT A TERTIARY HOSPITAL IN SOUTH AFRICA: A RETROSPECTIVE REVIEW

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Declaration

I Apamu Jacques Mapele, student number 3990882, declare that this Research Report is my own work. It is being submitted for the degree of Master at the university of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

Signature of candidate

12th day of June 2021 in Johannesburg

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Contents

Submissible Paper

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Abbreviations

ANC	Antenatal care
CMJAC	Charlotte Maxeke Johannesburg Academic Hospital
CONS	Coagulase-negative staphylococcus
CPAP	Continuous positive airway pressure
EONS	Early-Onset neonatal sepsis
ESBL	Extended Spectrum Beta Lactamase
CFR	Case fatality rate
CMV	Conventional Mechanical Ventilation
CRE	Carbapenem-resistant enterobacteriaceae
HIV	Human immuno-deficiency virus
LMICS	Low and middle income countries
LONS	Late onset neonatal sepsis
MAS	Meconium aspiration syndrome
NHLS	National Health Laboratory Service
NNS	Neonatal sepsis
NVD	Normal vaginal delivery
PROM	Premature rupture of membranes
RDS	Respiratory distress syndrome
SA	South Africa

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Abstract

Background. Approximately one third of under five deaths occur during the neonatal period. Neonatal sepsis (NNS) is major cause of morbidity and mortality.

Therefore, sick neonates with signs and symptoms of possible sepsis are provided with early empiric antibiotic therapy from birth. The choice of empiric antibiotic therapy is based on surveillance data of antimicrobial sensitivity patterns in culture isolates. Neonatal pathogens vary not only between different neonatal units but also over time in the same unit. The antibiotic susceptibility patterns of pathogens also change with time and with the emergence of multidrug resistant organisms.

The aim of this study were to describe clinical characteristics of neonates in a tertiary referral centre in Gauteng South Africa with culture confirmed blood stream infections as well as the causative organisms and their antimicrobial susceptibility patterns. This information will be used as part of Charlotte Maxeke Johannesburg Academic hospital (CMJAH) neonatal unit antimicrobial stewardship and guide empiric antibiotic therapy.

Methods. This was a descriptive retrospective study from January 2018 to June 2019. The clinical data of neonates with confirmed blood stream culture, and the susceptibility profile of organisms were reviewed.

Results. There were 386 neonates with positive blood stream culture, which represent a culture confirmed NNS incidence of 15.6 per 100 admissions. Late onset neonatal sepsis (LONS) represented the majority of NNS, 12.3 per 100 admissions (n=304). The commonly identified organism overall was *Coagulase-negative Staphylococcus* (CONS) (45.9%), followed by *Acinetobacter baumannii* (11.7%), *Staphylococcus aureus* (10.4%), *Klebsiella pneumoniae* (9.3%) and *Escherichia coli* (4.1%). The majority of *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli* were respectively methicillin-resistant *Staphylococcus aureus* (MRSA) (87.5%), extended spectrum beta-lactamase (ESBL) *Klebsiella pneumoniae* (88.9%), and ESBL *Escherichia coli* (56.3%). *Candida parapsilosis* (2.8%) was the predominant fungus, susceptible to amphotericin B.

Conclusion. NNS is a major problem in neonatal unit of CMJAH. The antibiotic resistant organisms are common. The commonly identified organism overall was CONS, followed by *Acinetobacter baumannii, Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli.* The suitable empiric antibiotic for early onset neonatal sepsis (EONS) would be ampicillin and amikacin while LONS would be covered by vancomycin and meropenem.

Background

South Africa (SA) is committed to reducing the under five mortality rate in line with the United Nation Sustainable Development Goal targets (1). The neonatal period accounts for nearly one-third of under five deaths, and neonatal sepsis (NNS) is a major cause of morbidity and mortality (1-3). NNS is defined as a clinical syndrome in an infant 28 days of life or younger (2). NNS is revealed by systemic signs of infection and isolation from blood stream of pathogen organism (2).

The potential for serious adverse outcomes is of such great consequence that the care givers should have a low threshold for evaluation and treatment for possible sepsis in neonates. Sick neonates with signs and symptoms of infection are therefore provided with early empiric antibiotic therapy from birth (2;3). The investigation of antimicrobial sensitivity configuration in culture isolates aligned the empiric antibacterial treatment (2;3). However, the overuse of antibiotics can result in the development of antimicrobial-resistant organisms including bacterial, fungal, and viral infections (3).

Neonatal pathogens vary not only between different neonatal units but also over time (2). Multidrug resistant organisms develop overtime (2;4). In many health care facilities, coagulase-negative staphylococcus (CONS) accounted for more than half of Gram-positive organisms which contribute up to 70% of hospital acquired infections in neonates (5). The Gram-positive pathogens are the commonest roots of infections than Gram negative pathogens and yeast (5). In lower and middle income countries (LMICS), Gram-negative organisms with a greater frequency of antimicrobial resistance can be far more predominant as neonatal pathogens (6). To guide the choice of empiric therapy, a constant observation of microbiological isolates and their susceptibility patterns is therefore an important part of antibiotic administration (3).

NNS is categorised according to the neonate age at the beginning of symptoms.

Early-onset neonatal sepsis (EONS) is specified as infection occurring within the first 72 hours of life (2). The morbidity and mortality related with EONS increases with the degree of prematurity, and identifying the risk factors for EONS and clinical conditions are useful in assessing for EONS (2). The organisms are usually transmitted by the mother (vertical transmission) (2). The organisms include: *Group B streptococcus, Escherichia coli, Staphylococcus aureus* (2;7;8).

Late onset neonatal sepsis (LONS) is the beginning of symptoms at more than 72 hours of age

(2). Organisms in this case are usually hospital acquired. The organism consists of: *Coagulase-negative Staphylococcus* (CONS), *Staphylococcus aureus*, *Multidrug-resistant Gram-negative rods* and *Candida* species (2). CONS is reported as the most prevalent organism isolated in LONS, and the rate of infection is on the rise in NNS (9;10). It has been revealed that CONS carried by neonatal personnel vary from those in the overall population (9). Neonatal personnel are possible source for cross-contamination of lethal CONS that originate from neonatal unit to patients (9). CONS has been related with long hospital stay, and indwelling medical devices (10). The most significant virulence feature of CONS is their biofilm-property (9;10).

The total incidence of culture confirmed NNS varies from one to five cases per 1000 live births depending on the case definition and the population studied (3). In LMICS, there is a wide variation in the incidence from one country to another and within a country there is variation between health care facilities. Authors in Pakistan have reported a rate of 5.6 per 1000 live births in a hospital based study compared to a rate of 54.9 per 1000 live births in a hospital in Nigeria (7;8). The incidence of NNS differs between the hospital and community based settings with a high incidence documented at the community level due to various reasons including poor hygiene condition and delayed presentation to hospital (11; 12). A retrospective study was carried out in the neonatal unit at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) from January to December 2012 and, there were 196 patients with blood-culture proven NNS (4). This led to an incidence of 10.326 per 100 admissions (4). LONS represented 83.5% of cases of culture confirmed NNS. Major pathogens were *Klebsiella pneumoniae* (32,2%), CONS (23,7%) and MRSA (13,13%) (4). Most of the identified *Klebsiella pneumoniae* were ESBL producing bacteria with resistance to ampicillin and gentamicin (4).

There is agreement amongst researchers that NNS carries a huge burden in LMICS and more research is needed to better understand this syndrome and shed light on its management (4). The aim of this study was to describe clinical characteristics of neonates in a tertiary referral centre in Gauteng South Africa with culture confirmed blood stream infections as well as the causative organisms and their antimicrobial susceptibility patterns. This information will be used as part of the unit's antimicrobial stewardship and guide empiric antibiotic therapy.

Methodology

Study design

This was a retrospective, descriptive study. The study population comprised all neonates admitted into the CMJAH with blood culture proven neonatal sepsis from January 2018 to June 2019.

Subjects

Study population

- All neonates admitted to CMJAH neonatal unit within 72 hours at birth

Inclusion criteria

- All neonates with confirmed blood stream infection.
- Neonates with corrected gestational age up to 28 days and confirmed blood stream culture.
- CONS was regarded as a pathogen and not a contaminant. (9;10)

Exclusion criteria

- Neonates with positive blood cultures with likely contaminants: Micrococcus spp, Corynebacterium spp, Streptococcus viridans, Pseudomonas fluorescens, Pseudomonas stutzeri, Actinomyces viscosus, Bacillus species and, Enterococcus casseliflavus (13).
- Neonates with suspected sepsis and negative blood stream cultures.
- Other infections, such as urinary tract infection, conjunctivitis, pneumonia, omphalitis, phlebitis, skin abscess and meningitis.
- Neonates who died in labour ward.
- Term neonates older than 28 days
- Neonates admitted to CMJAH neonatal unit after 72 hours of birth

Study site guidelines

Blood culture was collected on all neonates with suggestive signs and symptoms of NNS. The clinical signs and symptoms that suggest NNS include: temperature instability, distended abdomen, hyper or hypoglycaemia, large aspirate, irritability, lethargy, buldging fontanelle, tachypnoea, subcostal recession, nasal flaring. Neonates admitted with no clinical signs and symptoms suggestive of sepsis did not have blood cultures performed.

Every pathogenic organism identified was deemed as a distinct episode of positive blood stream infection, provided the cultures were taken more than seven days apart. Blood cultures samples were collected prior to antibiotic therapy initiation. After 72 hours of the antibiotic initiation, blood cultures were repeated if the previous one grew an organism otherwise the antibiotics were stopped immediately or 48 hours later if septic markers were suggestive or if there is no clinical improvement. If the blood culture grew a CONS, vancomycin was initiated due to occurrence of methicillin resistant CONS, and blood culture was repeated immediately if the clinical condition remains unchanged or deteriorate. If the repeated blood culture grew a CONS again, antibiotic therapy was continued and a source of infection was sought. If the repeat culture was negative the vancomycin was stopped after 7 days, provided that the clinical finding have improved. EONS was managed empirically with ampicillin and gentamicin through the study period. Empiric treatment for LONS was often started with meropenem and vancomycin, and could be changed in consultation with the microbiology unit in response to the evolving antibiograms prevalent in the unit at the time. Positive blood cultures were reviewed, and a joint decision was taken on the significance of the organism with microbiology unit and appropriate de-escalation of antibiotic treatment was introduced.

Staphylococcus aureus resistant to cloxacillin were defined as methicillin resistant *staphylococcus aureus* (MRSA). *Klebsiella pneumoniae* isolates resistant to cefotaxime /ceftriaxone were considered to be extended spectrum beta-lactamases (ESBL) producing *Klebsiella pneumoniae* and if they were resistant to carbapenem, they were noted as carbapenem-resistant Enterobacteriaceae (CRE).

Data management

Data was described separately for EONS and LONS. Data included patient characteristics as well as the identity and susceptibility of the causative organisms. Information including demographics, obstetric data and clinical characteristics were routinely collected for all neonates at the time of discharge for the purpose of clinical audit. Information included antenatal care, duration of hospitalization, gender, birth weight, HIV status, use of antenatal steroids, nasal continuous positive airway pressure (NCPAP), conventional mode of ventilation, broncho-pulmonary dysplasia (14), respiratory distress syndrome (RDS) (14), meconium aspiration pneumonia (MAS) (14), congenital pneumonia (14), necrotizing enterocolitis (NEC) Bell's stage 2 and 3 (15), retinopathy of prematurity (ROP) grades 3 and 4 (16), and intraventricular haemorrhage (IVH) grades 3 and 4(16).

Data was managed using Research Electronic Data Capture (REDCap) which was hosted by the University of the Witwatersrand (17). Patient information was routinely collected upon discharge from the neonatal unit. Data was reviewed twice before entry onto the custom REDCap database, and verified thereafter. Patient information was accessed from the neonatal database and culture results from the National Health Laboratory Service (NHLS) Trak-Care. The culture evidence consisted of identification of the organism isolated and the antibacterial susceptibility of every organism.

Study setting

CMJAH is a tertiary hospital with a neonatal unit of 104 beds capacity including paediatric and neonatal intensive care 14 beds, high and low care area 80 beds, and Kangaroo Mother care area 10 beds.

Data Analysis

Data was entered into a spreadsheet and basic statistical analysis was performed using SPSS version 25 (BMI, USA). Continuous data was represented as medians or means with standard deviations (SD), depending on the distribution. Categorical data was presented as percentages, and frequency.

Ethical consideration

The ethics clearance certificate number M190725 was issued by the Human Research Ethic Committee of the University of Witwatersrand. Data were de-identified (name, surname, hospital number and the date of birth were removed).

Results

Maternal demographic characteristics

The mean maternal age was 28.5 years (±5.12). Pre-eclampsia was the most common obstetric complication. See other maternal characteristics in Table 1.

Table 1. The Characteristics of mothers of neonates with positive blood cultures

Characteristic	**All cases (N=384),n(%)**
Maternal age (Years)	
< 18	56(14.6)
18 – 40	305(79.4)
> 40	23(6.0)
Antenatal care	221(57.6)
Steroids	109(28.4)
Chorioamnionitis	10(2.6)
Preeclampsia	82(21.4)
Vaginal delivery	191(49.7)
Caesarean section	193(50.3)
HIV positive	123(32.0)
RPR positive	6(1.6)

Neonatal clinical characteristics

The mean (±SD) birthweight was 1691.45 (±866.18) g. The mean (±SD) gestation age 29.6 weeks (±3.52) and mean (±SD) length of hospital stay was 30.98 days (±27.22). The most common neonatal diagnosis at the onset of sepsis was RDS (63.2%) and the most frequent respiratory therapy was nasal continuous positive pressure airway pressure (NCPAP) (56.0%). Neonates that required conventional mechanical ventilation (CMV), 33.1%(51/154) of them demised and their blood stream cultured grew mainly *Acinetobacter baumannii* (n=14), *Staphylococcus aureus* (n=10), *Klebsiella pneumonia* (n=8), *Coagulase-negative*

staphylococcus (n=8), *Candida parapsilosis* (n=4), *Streptococcus agalactiae* (n=3), *Candida albicans* (n=2), *Enterococcus faecalis* (n=1), and *Listeria monocytogenes* (n=1). Other characteristics of neonates are presented in Table 2.

Table 2. Demographic and problems list of neonates with proven positive blood culture

Demographic	**All neonatal cases (N=386),n(%)**
Male	204(52.8)
Birth weight (grams)	
<999	85(22.0)
1000 -1599	141(36.5)
1600 – 2499	86(22.3)
> 2500	74(19.2)
Feeds on discharge	
Breastmilk	171(44.3)
Formula	144(37.3)
Mixed feed	71(18.4)
HIV polymerase chain reaction positive	3(0.0)
Length of hospitalisation (days)	
< 10 days	116(30.0)
> 11 days	270(70.0)
Congenital pneumonia	11(2.8)
Respiratory distress syndrome	244(63.2)
Meconium aspiration syndrome	16(4.1)
Necrotizing enterocolitis (Bell's stage 2&3)	59(15.3)
Broncho-pulmonary dysplasia (> 28 days oygen)	15(3.9)
Intra-ventricular haemorrhage (Stage 3&4)	21(5.4)
Continuous positive airway pressure	216(56.0)
Conventional mechanical ventilation	154(39.9)
Retinopathy of prematurity (Stage 3&4)	12(3.1)

Organisms isolated

During the study period, the neonatal unit admitted 2465 neonates. There were 386 neonates with positive blood stream cultures including two sets of twins, which represents a culture proven NNS incidence of 15.6 per 100 admissions. There were no repeat cultures within a 7-days period with the same pathogen for any of the neonates. Blood culture confirmed LONS represented the majority of NNS, 12.3 per 100 admissions (n=304). The incidence of blood culture confirmed EONS was 3.3 per 100 admissions (n= 82). The most common isolated organisms as blood culture confirmed EONS were CONS (58.5%) and *Streptococcus agalactiae* (7.3%), and as blood culture confirmed LONS was CONS (42.4%) followed by *Acinetobacter baumannii* (13.2%) (Table 3). There were 29.5% (114/386) neonates with established positive blood stream culture who died. The majority of deaths were from *Acinetobacter baumannii* with a case fatality rate (CFR) of 73.3% (33/45). Followed by *Staphylococcus aureus, Klebsiella pneumonia* and *Coagulase-negative staphylococcus* with respective CFR of 75.0% (30/40), 50.0% (18/36) and 7.9% (14/177).

Table 3. Organisms isolated in early onset and late onset sepsis in neonates

Organism	Neonatal sepsis (N= 386),n(%)	EONS (N= 82),n(%)	LONS (N= 304),n(%)
Gram-positive	**247(64.0)**	**64(78.0)**	**183(60.2)**
Coagulase-negative staphylococcus	177(45.9)	48(58.5)	129(42.4)
Staphylococcus aureus	40(10.4)	5(6.1)	35(11.5)
Streptococcus agalactiae	9(2.3)	6(7.3)	3(1.0)
Streptococcus pneumoniae	3(0.8)	2(2.4)	1(0.3)
Enterococcus faecalis	9(2.3)	1(1.2)	8(2.6)
Enterococcus faecium	7(1.8)	0(0.0)	7(2.3)
Listeria monocytogenes	2(0.5)	2(2.4)	0(0.0)
Gram-negative	**123(31.9)**	**18(22.0)**	**105(34.5)**
Enterobacteriaceae			
Citrobacter species	2(0.5)	0(0.0)	2(0.7)
Escherichia coli	16(4.1)	1(1.2)	15(4.9)
Enterobacter species	7(1.8)	0(0.0)	7(2.3)
Klebsiella pneumoniae	36(9.3)	6(7.3)	30(9.9)
Morganella morganii	2(0.5)	0(0.0)	2(0.7)
Serratia marcescens	3(0.8)	0(0.0)	3(1.0)
Non- fermenters			
Acinetobacter baumannii	45(11.7)	5(6.1)	40(13.2)
Elizabethkingia meningoseptica	1(0.3)	0(0.0)	1(0.3)
Pseudomonas aeruginosa	2(0.5)	1(1.2)	1(0.3)
Stenotrophomonas maltophilia	2(0.5)	0(0.0)	2(0.7)
Achromobacter xylosoxidans	1(0.3)	1(1.2)	0(0.0)
Other			
Haemophilus parainfluenzae	6(1.6)	4(4.9)	2(0.7)
Fungi	**16(4.1)**	**0(0.0)**	**16(5.3)**
Candida albicans	4(1.0)	0(0.0)	4(1.3)
Candida parapsilosis	11(2.8)	0(0.0)	11(3.6)
Candida utilis	1(0.3)	0(0.0)	1(0.3)

EONS= Early onset neonatal sepsis, LONS= Late onset neonatal sepsis

Susceptibility profiles

Tables 4 to 6, show the susceptibility results for the frequently isolated bacteria in the study.

Table 4. Susceptibility results for the Gram-positive organisms

Antibiotics	**Bacteria**											
	CONS n = 177		**STAU n = 40**		**STRAG n= 9**		**STRPN n= 3**		**ENCFA n= 9**		**ENCFE n= 7**	
	NT	SS(%)	NT	SS(%)	NT	SS	NT	SS(%)	NT	SS(%)	NT	SS(%)
Ampicillin/Penicillin	106	6(6)	40	2(5)	9	9(100)	3	2(67)	8	7(88)	7	0(0)
Cefotaxime/ceftriaxone	0		0		0		3	3(100)	0		0	
Cloxacillin	102	15(15)	40	15(38)	0		0		0		0	
Vancomycin	108	108(100)	40	40(100)	9	9(100)	3	3(100)	8	8(100)	7	7(100)

NT = number tested; SS = number of susceptible strains, CONS = *Coagulase negative Staphylococcus*, STAU = *Staphylococcus aureus*, STRAG= *Streptococcus agalactiae*, STRPN = *Streptococcus pneumoniae*, ENCFA = *Enterococcus faecalis*, ENCFE = *Enterococcus faecium*

Susceptibility testing was done in 98% of organisms. The susceptibility of all the Gram-positive organisms to ampicillin/penicillin and vancomycin was tested. All CONS, *Staphylococcus aureus and Enterococcus* species were susceptible to vancomycin (Table 4). The Gram-negative organisms were subdivided into *Enterobacteriaceae,* non-fermenters, and other organisms. All isolated *Klebsiella* species were susceptible to amikacin (100%). ESBL *Klebsiella* represented 88.9% (n=32/36) and CRE *Klebsiella* 0.6% *(*n=2/36) of all *Klebsiella* species. All the other *Enterobacteriaceae (Escherichia coli, Enterobacter* species, *Citrobacter* species, *Serratia marcescens*) were sensitive to amikacin, and meropenem (Table 5). The *Escherichia coli* ESBL rate was 56.3% (n=9/16) (Table 5).

Table 5. Susceptibility of the Enterobacteriaceae

Antibiotics	Bacteria											
	KLEPN n = 36		**E. Coli n= 16**		**ENTSP n= 7**		**CITSP n= 2**		**SERMA n= 3**		**MORMO n= 2**	
	NT	SS(%)	NT	SS(%)	NT	SS(%)	NT	SS(%)	NT	SS(%)	NT	SS(%)
Penicillin/ Ampicillin	0		16	4(25)	0		0		0		0	
Cefotaxime/ Ceftriaxone	36	4(11)	16	7(44)	7	6(86)	2	2(100)	3	3(100)	2	1(50)
Ceftazidime	36	4(11)	16	15(94)	7	6(86)	2	2(100)	3	3(100)	2	1(50)
Cefepime	36	11(31)	16	11(69)	7	6(86)	2	2(100)	3	3(100)	2	1(50)
Meropenem	36	34(94)	16	16(100)	7	7(100)	2	2(100)	3	3(100)	2	1(50)
Imipenem	36	34(94)	16	16(100)	7	7(100)	2	2(100)	3	0(0)	2	1(50)
Gentamicin	36	4(11)	16	13(81)	7	6(86)	2	2(100)	3	3(100)	2	2(100)
Amikacin	36	36(100)	16	16(100)	7	7(100)	2	2(100)	3	3(100)	2	2(100)
Tobramycin	2	0(0)	1	0(0)	7	0(0)	2	0(0)	3	0(0)	2	0(0)

NT = number tested; SS = number of susceptible strains, KLEPN = *Klebsiella pneumonia*, E. Coli= *Escherichia coli*, ENTSP = *Enterobacter* species, CITSP = *Citrobacter* species, SERMA = *Serratia marcescens*, MORMO = *Morganella morganii*

Isolated *Acinetobacter baumannii* (ACIBA) *and Pseudomonas aeruginosa* (PSEAE) were 100% sensitive to colistin. Other tested antibiotics sensitivity were not as effective as colistin to both organisms (Table 6).

Table 6. Sensitivity of the isolated *Acinetobacter baumannii* and *Pseudomonas aeroginosa*

Antibiotics	**Bacteria**			
	ACIBA (n= 45)		**PSEAE (n= 2)**	
	NT	SS(%)	NT	SS(%)
Ceftazidime	45	6(13)	2	1(50)
Cefepime	45	5(11)	2	2(100)
Meropenem	45	4(9)	2	1(50)
Imipenem	45	5(11)	2	1(50)
Gentamicin	44	3(7)	2	1(50)
Amikacin	39	2(5)	2	2(100)
Ciprofloxacin	45	7(16)	2	1(50)
Piperacillin/tazobactam	45	3(7)	2	2(100)
Tobramycin	45	1(2)	2	1(50)
Tigecycline	45	42(93)	0	
Colistin	41	41(100)	2	2(100)

ACIBA = *Acinetobacter baumannii*, PSEAE = *Pseudomonas aeruginosa*, NT = number tested, SS= number of susceptible strains

All *Candida isolates* (*albicans, parapsilosis,* and *utilis)* were susceptible to amphotericin B. *Candida utilis* was only tested to amphotericin B. *Candida albicans* was 100% susceptible to fluconazole and micafungin. *Candida parapsilosis* was the predominant fungal sepsis isolate with 100% susceptible to micafungin and 18% susceptibility to fluconazole.

Discussion

In 2012 the blood culture proven NNS incidence in our unit was 10.3% (196/1903) (4). While in this study the same incidence of culture confirmed NNS has increased to 15.6% (386/2465). The preponderance of Gram-positive organisms as the aetiology of NNS in the CMJAH neonatal unit has been noted. The high proportion of Gram-positive organisms causing NNS is in keeping with Patel SJ et al in the United States of America (5), contrary to the findings of surveillance studies in our neonatal unit and other LMICS, where Gram-negative organisms were found to be the major relevant agents in NNS (4,18). It should be mentioned that this comparative study, CONS were included (4,5,18).

The commonly identified organism overall was CONS, followed by *Acinetobacter baumannii, Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli.*

The high proportion of CONS (45.9%) in this study differed with the retrospective study conducted in neonatal unit at CMJAH from January to December 2012 and other LMICS (4,19). Nonetheless, this may be attributed to CONS being commonly omitted from study, as it is often judged to be a contaminant (19). However, CONS is a pathogen in neonates, immunosuppressed persons and neonates with foreign bodies. As cause of NNS, the significance of CONS has been described elsewhere (9;10). Furthermore, it has been revealed that CONS carried by NICU employees vary from those in overall population (9). In VLBW neonates, the danger of CONS infection surge considerably due to mechanical ventilation, parenteral nutrition, central lines and greater exposer to invasive skin or mucosa penetrating procedures creates their biofilm forming capacities and higher rate of antibiotic resistance provides them a selective benefit over other organisms (9,10). CONS is more likely to cause NNS. This might be contrary to other studies that virulent CONS are acquired on NICU personnel, normal skin commensal and are likely to cause cross contamination (9). In eight neonatal units Australia, a one-year retrospective study stated that CONS was the commonest isolated organism (18). The prevalence study of NNS established on eight selected articles issued in 2016 and 2017 with facts on demographics, bacteria distribution, risk factors, antibacterial susceptibility and increasing isolation of CONS among other bacterial isolates (20). The major bacterial isolate was CONS and it accounted 66.6% of NNS (20). Referring to a laboratory-based retrospective study piloted in 47 NICU in France, CONS was found in 43 NICU (91.5%) and occurring as 46% of all positive blood culture (21)

There has been a rise of *Acinetobacter baumannii* identified in the unit. In 2012, a study by Ballot et al (3), reported the prominence of *Acinetobacter baumannii* as a source of NNS in the unit, while the organism represented 10% of bacterial sepsis isolated in comparison with the prior study in the same unit 2006/2007 where this organism was not even isolated. In 2017, Lebea et al (4), noted in a retrospective study that *Acinetobacter baumannii* accounted 9.2% of NNS. We found that this organism is the commonest Gram-negative and it accounted 11.7% of NNS.

There has been a decline in the proportion of *Klebsiella pneumoniae* in our unit. In 2017 Lebea et all indicated that 97.3% of *Klebsiella pneumonia* were ESBL producing strains compared with 88.9% in this study (4). However, this proportion of ESBL *Klebsiella pneumoniae* remain high compare to the 2012 study reported by Ballot et all in our unit where the proportion was 70.8% (3). There remains significant room for improvement.

Acinetobacter baumannii and ESBL *Klebsiella pneumonia* were not susceptible to beta-lactam antibiotics, third-generation cephalosporin and to other drugs classes for instance aminoglycosides. In resource constrained settings with restricted treatment options, these bacteria represent a huge challenge.

Staphylococcus aureus is also declining as a cause of NNS in our unit, accounting for 10.4% of NNS. Compared to the previous study in 2017 where *Staphylococcus aureus* accounted for 14.3% (3.4). But the unit should improve since in 2012 study in the same unit, *Staphylococcus aureus* accounted for 8.3% of NNS (3). We found that most *Staphylococcus aureus* were MRSA (62.5%), which is very high compare to the previous study (13.1%) in 2017 (4). All these *Staphylococcus aureus species* remained susceptible to vancomycin as previously noted (22).

NNS is currently a global issue with the rise of resistant organisms (18). Furthermore, growing resistance to frequently use antibiotics has been established in this study. Most of identified CONS, *Acinetobacter baumannii, Klebsiella pneumoniae* and *Staphylococcus aureus* were not susceptible to the first line antibiotic agents used in EONS namely ampicillin and gentamicin.

Meropenem and vancomycin were used as first-line agents for treatment of LONS. All of the CONS and *Staphylococcus aureus* were susceptible to vancomycin, while *Klebsiella pneumoniae* to meropenem. Only 9% of the *Acinetobacter baumannii* isolates were susceptible to meropenem. Hence the majority of the *Acinetobacter baumannii* sepsis cases required treatment with colistin. It is alarming that the predominant Gram-negative pathogen in this unit is extremely drug resistant and is largely not covered by the empiric Antibiotic used.

Amphotericin B were used as empiric antifungal therapy in the unit. Candida parapsilosis was the predominant fungus in this study and susceptible to amphotericin B, in keeping with the previous study in this unit (23).

Conclusions

NNS is a major problem in the neonatal unit of CMJAH and antibiotic resistant organisms are common. The commonly identified organism overall was CONS, followed by *Acinetobacter baumannii, Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli*. Based on the sensitivity profiles in the current study, the appropriate empiric antibiotic for EONS would be ampicillin and amikacin while LONS would be covered by vancomycin and meropenem. The suitable empiric antifungal therapy is amphotericin B. Antibiotic therapy must be tailored to the organism antimicrobial sensitivity in consultation with microbiology unit. Similarly, empiric therapy should be discontinued as soon as sepsis is ruled out. Constant antimicrobial surveillance is vital and obedience to infection control measures, mainly hand washing, as part of antibiotic stewardship to curb down antibiotic resistance.

Study limitations

Viral pathogens were not routinely identified. Negative blood stream cultures to septic neonates were excluded and it is possible that same of these neonates were infected. The risk factors for NNS were not looked into, such as central catheter and urinary catheter. Clinical presentations and septic markers were also not included in the current study. Due to the retrospective nature of the study, investigators could not assess the manner blood cultures were taken, such as antiseptic procedure and suitable volumes of blood. Also due to the retrospective nature of the study, missing or unknown information on the database could not be retrieved.

Conflicts of interest

The authors confirm no competing interests.

Funding

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Approved Protocol

AN AUDIT OF CULTURE-PROVEN NEONATAL SEPSIS AT A TERTIARY HOSPITAL IN SOUTH AFRICA: A RETROSPECTIVE REVIEW

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ABBREVIATIONS

ANC	:	Antenatal natal care
C/section	:	Caesarean section
CMJH	:	Charlotte Maxeke Johannesburg Academic Hospital
CPAP	:	Continuous positive airway pressure
EONS	:	Early-Onset neonatal sepsis
ESBL	:	Extended beta Lactamase
IPPV	:	Intermittent positive airway pressure
IVH	:	Intra ventricular haemorrhage
LMICS	:	lower& middle income countries
LONS	:	Late onset neonatal sepsis
MAS	:	Meconium aspiration syndrome
NHLS	:	National Health Laboratory Service
NNS	:	Neonatal sepsis
NVD	:	Normal vaginal delivery
PROM	:	Premature rupture of membranes
RDS	:	Respiratory distress syndrome
ROP	:	Retinopathy of prematurity
RVDexp/unexp		Retroviral disease exposed / Unexposed
SA	:	South Africa
WCC	:	White count cell

I. Background

South Africa (SA) is committed to reducing under five mortality rate in line with the Sustainable Development Goal targets (1). Approximately one-third of under five deaths occur during the neonates period, and neonatal sepsis (NNS) is a major cause of morbidity and mortality (1). The risk factors and clinical outcomes of sepsis are poorly understood (2). NNS is defined as a clinical syndrome in an infant 28 days of life or younger, manifested by systemic signs of infection and isolation of a bacterial pathogen from blood stream (3).

The potential for serious adverse outcomes is of such great consequence that the care giver should have a low threshold for evaluation and treatment for possible sepsis in neonates. Sick neonates are therefore provided with early empiric antibiotic from birth (4). Empiric antibiotic therapy is based on surveillance of antimicrobial sensitivity patterns in culture isolates (5). Prolonged early antibiotic exposure has been associated with a subsequent increased risk of necrotizing enterocolitis (NEC) and death, even when adjusted for gestational age and initial severity of illness (5). Overuse of antibiotics results in the development of antimicrobial-resistant organisms (5). Faced with a patient population for whom both the administration of and the withholding of antibiotic may each have life threatening consequences, neonatal clinicians are challenged to identify those babies at low enough risk of sepsis to minimize antibiotic exposures.

Neonatal pathogens vary not only between different neonatal units but also over time in the same unit. The antibiotic susceptibility of organisms also changes with time, with the emergence of multidrug resistant organisms (6). In many health care facilities, gram-positive organisms cause up to 70% of nosocomial infections in neonates (7) with coagulase-negative staphylococcus (CONS) accounting for more than half of these (8). In Lower and middle in countries (LMlCS), gram-negative organisms may be far more prevalent as neonatal pathogens, with a higher incidence of antimicrobial resistance (9). Ongoing surveillance of microbiological isolates and their sensitivity patterns is therefore an essential part of antibiotic stewardship, in particular to guide the selection of empiric therapy (5).

Risk factors for NNS include:

A. Maternal factors: Chorioamnionitis, maternal group B streptococcal colonization, maternal urinary tract infection (UTl), multiple pregnancies, premature rupture of membranes(PROM), preterm delivery (<37 weeks), prolonged rupture of membranes (10; 11)

B. Neonatal host factors: Breakage of the natural barriers (skin and mucosa), invasive procedures like endotracheal intubation, NEC, prolonged use of antibiotics, prolonged indwelling catheter uses, H2-receptor blocker or proton pump inhibitor use, decreased transfer of maternal immunoglobulin and specific antibody, immature function of immune system (10; 11)

C. Virulence of colonizing organism.

NNS is classified according to the infant's age at the onset of symptoms.

Early-onset neonatal sepsis (EONS) is defined as infection occurring within the first 72 hours of life (2). The morbidity and mortality associated with EONS increases with the degree of prematurity.

Identifying the risk factors for EONS and new-born clinical condition are useful in assessing for EONS (5). The organisms are usually transmitted by the mother (vertical transmission). The infection is caused by bacterial, fungal, and viral infections (Table 1).

Late-onset neonatal sepsis (LONS) is defined as onset of symptoms at more than 72 hours of age. Organisms in this case come usually from the environment (nosocomial, Table 1) (12; 13)

Table 1: Organisms associated with early-onset and late-onset neonatal sepsis

Early-onset sepsis	Late-onset sepsis
Group B streptococcus	*Coagulase-negative Staphylococcus*
Escherichia coli	*Staphylococcus aureus*
Listeria monocytogenes	*Enterococcï*
Other *Streptococci*: *Streptococcus pyogenes, viridans group streptococci, Streptococcus pneumoniae*	Multidrug-resîstant gram-negative rods (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas, Enterobacter, Citrobacter, Serratia species)*
Enterococci	*Candida species*
Nontypable Haemophilus influenzae	

Signs and symptoms of NNS are often non-specific, however increased respiratory rate, heart rate, baby response to stimuli, and the colour of the umbilicus are important parameters (14). The progression of the septic process and the risks of adverse outcome increase as follows: Organism inoculation leads to focal infection or bacteraemia, followed by sepsis, sepsis syndrome, early septic shock, refractory septic shock, multiple organ dysfunction syndrome and death (15).

The overall incidence of NNS ranges from one to five cases per 1000 live births. Estimated incidence rates vary based on the case definition and the population studied. In LMICS, there is a wide variation in the incidence from one country to another and within a country there is variation between health care facilities. Authors in Pakistan have reported a rate of 5.6 per 1000 live births in a hospital based study compared to a rate of 54.9 per 1000 live births in a hospital in Nigeria (16;17). The incidence of NNS differs between the hospital and community based study with a high incidence documented at the community level due to various reasons including poor access to health care facilities and delayed presentation to hospital (18; 19). A retrospective study was conducted in neonatal unit at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) from January to December 2012, there were 196 patients with blood-culture proven NNS in CMJAH (6). This gave an incidence of 10.326 per 100 admissions (6). LONS accounted for 83.5% of cases of neonatal sepsis. Predominant pathogens were *KlebsÎella pneumoniae* (32,2%), CONS (23,7%) and MRSA (13,13^{0}/0) (6). Most of the identified

Klebsiella pneumoniae were ESBL producing bacteria with resistance to ampicillin and gentamicin (6).

There is an agreement among researchers that NNS carries a huge burden in I-MIC and more research is needed to better understand this syndrome and shed light on its management (6). The aim of our study is to describe neonates in a tertiary referral centre in Gauteng South Africa with culture proven sepsis. This information will be used as part of the unit's antimicrobial stewardship and guides empiric antibiotic therapy.

2.0bjectlves

1.To describe neonates admitted to neonatal unit with culture proven sepsis.

2. To describe the causative organisms.

3.methodology

Study design

This is a retrospective, descriptive study. The study population will comprise all neonates admitted into the CMJAH with culture proven neonatal sepsis from January 2018 to June 2019.

Subjects

1. Study population

- All neonates admitted to CMJAH neonatal unit within 72 hours at birth.

2. Inclusion criteria

- All neonates with blood stream infection.

3. Exclusion criteria

- Neonates with contaminants positive blood culture: *Micrococcus spp, Corynebacterium spp* and *Streptococcus viridans.*
- Neonates who died in labour ward.

Data collection

Data will be described separately for EONS and LONS. This will include patient characteristics and outcome, as well as the identity and susceptibility of the causative organisms. Information including demographics, obstetric data, clinical and outcome is collected for clinical audit on discharge of each neonate. Data is managed using Research Electronic Data Capture (Redcaps) which is hosted by the university of the Witwatersrand (20).

Patient information will be obtained from the database and culture results from the National Health Laboratory Service (NHLS) Track Care.

Data Analysis

Data collected will be entered into a spreadsheet and basic statistical analysis will be performed using IBM SPSS version 25. Continuous data will be described as medians or means with standard deviations, depending on the distribution categorical data will be presented as percentages, and frequency.

Limitations of the study

- Viral pathogens are not routinely identified.
- Neonates with clinical sepsis but negative cultures are excluded and it is possible same of these babies were infected.
- Some information like central catheter and antibiotic use was not collected

Definitions

- Neonates with single significant isolated organism in two different positive blood culture within seven days will be consider as single episode of sepsis.
- Intraventricular haemorrhage III & IV(IVH): blood within ventricular system with distension (grade Ill). Intra-ventricular and parenchyma' bleed (echo density) (grade IV) (21)
- Bronchopulmonary dysplasia (BPD) is persistent oxygen requirement up to 36 weeks postmenstrual age in preterm infants or up to 28 days in term infants with abnormal chest X-rays (21).
- Retinopathy of prematurity(ROP) stage Ill & IV: is a vision threatening disease associated with abnormal retinal vascular development that occurs only in prematurity. Partial retinal detachment (stage Ill) and total retinal detachment (stage IV) (22).
- Respiratory distress syndrome (RDS) is primarily a disorder of surfactant deficiency resulting in pulmonary insufficiency from soon after birth (23).
- Pneumonia is an infection that inflames the air sacs in one or both lungs
- Meconium aspiration syndrome (MAS) is respiratory distress in a newborn who has breathed (aspirate) a dark green, sterile faecal material called meconium into the lungs before or around the time of birth.

- Necrotizing enterocolitis (NEC) modified Bell's staging (24)

Stage	Clinical	Laboratory	Radiological	Management
lla	Temperature instability, apnoea, bradycardia, Decrease SaO2, increase gastric residuals, abdominal distension, stool-occult blood+ve, decreased or absent bowel sound, guarding	Mild metabolic acidosis, mild thrombocytopenia	Dilated bowel loops, sentinel loops, pneumatosis intestinally	NPO, gastric decompression, X-ray abdomen 6-8 hourly (AP &lateral), inform surgeon. Antibiotics for 7-10 days after septic work up.
IIb	Stage lla absent bowel sounds, marked guarding, abdominal wall discoloration or cellulitis,	Metabolic acidosis, thrombocytopenia, hyponatremia	lla + portal venous gas shadows	Same as above + supportive treatment

	right lower quadrant mass			
IIIa	Stage hypotension, DIC	Respiratory acidosis, metabolic acidosis, neutropenia, PT/PTT/INR, dimer, Increased CRP	Stage Il + ascites	Same as above insure adequate coverage for gram negative bacteria anaerobes
IIIb	Same as above	Same as above	stage & Ilia + pneumoperitoneum	Above emergency surgery

4.ethic

The research protocol will be submitted to the Human Research Ethic Committee of the University of Witwatersrand for approval before any data collection. Data will be de-identified (name, surname, hospital number and the date of birth will be removed) and the investigator will separate the key password protected. This will ensure no violations of patient rights and the study is conducted in an ethical manner. The information obtained from the study will be used to benefit neonates and mothers admitted to CMJAH in the future and form part of database that can be used for future research that will improve how we provide health care service to our patients.

5.timeline

	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JAN	FEB
Protocol development											
Ethic											
Data collection											
Data analysis											
Write up											
Submission											

6.fundlng

The costs of the study are anticipated to be minimal. A budget of R1300 is proposed for transport, stationary and printing which will be provided by the researcher.

DATA COLLECTION SHEET

STUDY NUMBER:

I.MATERNAL DATA

MATERNAL AGE:

VARIABLE	YES	NO	UNKNOWN
BOOKED			
STEROIDS			
CHORIOAMINONITIS			
PRECLAMPSIA			
VAGINAL DELIVERY			
C/ SECTION			
HIV positive			
RPR positive			

2.NEONATES DATA

SEX: MALE GESTATIONAL AGE:

FEED ON DISCHARGE: BREASTMILK FORMULA MIXED FEED

RVD EXPOSED

Y	N

HIV P N

AGE AT PRESENTATION:

Birth Weight: g

PROBLEM LIST	YES	NO
PNEUMONIA		
RDS		
MAS		
NEC(Grade 2&3)		
28 days)		
IVH (Grade 3&4)		
CPAP		
IPPV		
LENGTH OF HOPITALISATION		
ROP (Stage 3&4)		
DEATH		

GRAM NEGATIVE						
PATHOGENE ID		EONS	LONS		EONS	LONS
AMPICILLIN						
CEFOTAXIM						
CEFTRIAXON						
GENTAMICIN						
AMIKACIN						
MEROPENEM						
ERTAPENEM						
COLISTIN						
PIPTAZ						
CIPROFLOXACIN						
GRAM POSITIVE						
PATHOGENE ID		EONS	LONS		EONS	LONS
AMPICILLIN						
CEFOTAXIM						
CEFTRIAXON						
CLOXACILLIN						
VANCOMYCIN						
CEFTAZIDIN						
OTHERS						
PATHOGENE ID		EONS	LONS		EONS	LONS
AMPHOTERICIN						
FLUCONAZOL						

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SAMJ Author Guidelines

Manuscript preparation for South African Medical Journal

General article format/layout

Accepted manuscripts that are not in the correct format specified in these guidelines will be returned to the author(s) for correction, which will delay publication.

General:

- Manuscripts must be written in UK English.
- The manuscript must be in Microsoft Word format. Text must be single-spaced, in 12-point Times New Roman font, and contain no unnecessary formatting (such as text in boxes).
- Please make your article concise, even if it is below the word limit.
- Qualifications, ***full*** affiliation (department, school/faculty, institution, city, country) and contact details of ALL authors must be provided in the manuscript and in the online submission process.
- Abbreviations should be spelt out when first used and thereafter used consistently, e.g. 'intravenous (IV)' or 'Department of Health (DoH)'.
- Include sections on Acknowledgements, Conflict of Interest, Author Contributions and Funding sources. If none is applicable, please state 'none'.
- Scientific measurements must be expressed in SI units except: blood pressure (mmHg) and haemoglobin (g/dL).
- Litres is denoted with an uppercase L e.g. 'mL' for millilitres).
- Units should be preceded by a space (except for % and ºC), e.g. '40 kg' and '20 cm' but '50%' and '19ºC'.
- Please be sure to insert proper symbols e.g. µ not u for micro, a not a for alpha, b not B for beta, etc.
- Numbers should be written as grouped per thousand-units, i.e. 4 000, 22 160.
- Quotes should be placed in single quotation marks: i.e. The respondent stated: '...'
- Round brackets (parentheses) should be used, as opposed to square brackets, which are reserved for denoting concentrations or insertions in direct quotes.
- If you wish material to be in a box, simply indicate this in the text. You may use the table format –this is the *only* exception. Please DO NOT use fill, format lines and so on.

SAMJ is a generalist medical journal, therefore for articles covering genetics, it is the responsibility of authors to apply the following:

- Please ensure that all genes are in italics, and proteins/enzymes/hormones are not.

- Ensure that all genes are presented in the correct case e.g. TP53 not Tp53.

**NB: Copyeditors cannot be expected to pick up and correct errors wrt the above, although they will raise queries where concerned.

- Define all genes, proteins and related shorthand terms at first mention, e.g. '188del11' can be glossed as 'an 11 bp deletion at nucleotide 188.'

- Use the latest approved gene or protein symbol as appropriate:

- Human Gene Mapping Workshop (HGMW): genetic notations and symbols
- HUGO Gene Nomenclature Committee: approved gene symbols and nomenclature
- OMIM: Online Mendelian Inheritance in Man (MIM) nomenclature and instructions

- Bennet et al. Standardized human pedigree nomenclature: Update and assessment of the recommendations of the National Society of Genetic Counsellors. J Genet Counsel 2008; 17:424-433: standard human pedigree nomenclature.

Preparation notes by article type

Research

Guideline word limit: 4 000 words

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

Select figures and tables for your paper carefully and sparingly. Use only those figures that provided added value to the paper, over and above what is written in the text.

Do not replicate data in tables and in text.

Structured abstract

- This should be 250-400 words, with the following recommended headings:
  - **Background:** why the study is being done and how it relates to other published work.
  - **Objectives:** what the study intends to find out

- **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.
- **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
- **Conclusion:** must be supported by the data, include recommendations for further study/actions.

- Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors.
- Do not include any references in the abstracts.

Main article

All articles are to include the following main sections: Introduction/Background, Methods, Results, Discussion, Conclusions.

The following are additional heading or section options that may appear within these:

- Objectives (within Introduction/Background): a clear statement of the main aim of the study and the major hypothesis tested or research question posed
- Design (within Methods): including factors such as prospective, randomisation, blinding, placebo control, case control, crossover, criterion standards for diagnostic tests, etc.
- Setting (within Methods): level of care, e.g. primary, secondary, number of participating centres.
- Participants (instead of patients or subjects; within Methods): numbers entering and completing the study, sex, age and any other biological, behavioural, social or cultural factors (e.g. smoking status, socioeconomic group, educational attainment, co-existing disease indicators, etc) that may have an impact on the study results. Clearly define how participants were enrolled, and describe selection and exclusion criteria.
- Interventions (within Methods): what, how, when and for how long. Typically for randomised controlled trials, crossover trials, and before and after studies.
- Main outcome measures (within Methods): those as planned in the protocol, and those ultimately measured. Explain differences, if any.

Results

- Start with description of the population and sample. Include key characteristics of comparison groups.
- Main results with (for quantitative studies) 95% confidence intervals and, where appropriate, the exact level of statistical significance and the number need to treat/harm. Whenever possible, state absolute rather than relative risks.

- Do not replicate data in tables and in text.
- If presenting mean and standard deviations, specify this clearly. Our house style is to present this as follows:
- E.g.: The mean (SD) birth weight was 2 500 (1 210) g. Do not use the ± symbol for mean (SD).
- Leave interpretation to the Discussion section. The Results section should just report the findings as per the Methods section.

Discussion

Please ensure that the discussion is concise and follows this overall structure – sub-headings are not needed:

- Statement of principal findings
- Strengths and weaknesses of the study
- Contribution to the body of knowledge
- Strengths and weaknesses in relation to other studies
- The meaning of the study – e.g. what this study means to clinicians and policymakers
- Unanswered questions and recommendations for future research

Conclusions

This may be the only section readers look at, therefore write it carefully. Include primary conclusions and their implications, suggesting areas for further research if appropriate. Do not go beyond the data in the article.

Illustrations/photos/scans

- If illustrations submitted have been published elsewhere, the author(s) should provide consent to republication obtained from the copyright holder.
- Figures must be numbered in Arabic numerals and referred to in the text e.g. '(Fig. 1)'.
- Each figure must have a caption/legend: Fig. 1. Description (any abbreviations in full).
- All images must be of high enough resolution/quality for print.
- All illustrations (graphs, diagrams, charts, etc.) must be in PDF or jpeg form.

- Ensure all graph axes are labelled appropriately, with a heading/description and units (as necessary) indicated. Do not include decimal places if not necessary e.g. 0; 1.0; 2.0; 3.0; 4.0 etc.
- Scans/photos showing a specific feature e.g. *Intermediate magnification micrograph of a low malignant potential (LMP) mucinous ovarian tumour. (H&E stain).* –include an arrow to show the tumour.
- Each image must be attached individually as a 'supplementary file' upon submission (not solely embedded in the accompanying manuscript) and named Fig. 1, Fig. 2, etc.

Tables

- Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged.
- Large tables will generally not be accepted for publication in their entirety. Please consider shortening and using the text to highlight specific important sections, or offer a large table as an addendum to the publication, but available in full on request from the author
- Embed/include each table in the manuscript Word file - do not provide separately as supplementary files.
- Number each table in Arabic numerals (Table 1, Table 2, etc.) and refer to consecutively in the text.
- Tables must be cell-based (i.e. not constructed with text boxes or tabs) and editable.
- Ensure each table has a concise title and column headings, and include units where necessary.
- Footnotes must be indicated with consecutive use of the following symbols: * † ‡ § ¶ || then ** †† ‡‡ etc.

Do not: Use [Enter] within a row to make 'new rows':

Rather:

Each row of data must have its own proper row:

Do not: use separate columns for *n* and %:

Rather:

Combine into one column, *n* (%):

Do not: have overlapping categories, e.g.:

Rather:

Use <> symbols or numbers that don't overlap:

References

NB: *Only complete, correctly formatted reference lists in Vancouver style will be accepted. Reference lists must be generated manually and not with the use of reference manager software. Endnotes must* ***not*** *be used.*

- Authors must verify references from original sources.
- Citations should be inserted in the text as superscript numbers between square brackets, e.g. These regulations are endorsed by the World Health Organization, [2] and others. [3,4-6]
- All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order).
- Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus.
- Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al.
- Volume and issue numbers should be given.
- First and last page, in full, should be given e.g.: 1215-1217 **not** 1215-17.
- Wherever possible, references must be accompanied by a digital object identifier (DOI) link). Authors are encouraged to use the DOI lookup service offered by CrossRef:
  - On the Crossref homepage, paste the article title into the 'Metadata search' box.
  - Look for the correct, matching article in the list of results.
  - Click Actions > Cite
  - Alongside 'url =' copy the URL between { }.
  - Provide as follows, e.g.: https://doi.org/10.7196/07294.937.98x

Some examples:

- *Journal references:* Price NC, Jacobs NN, Roberts DA, et al. Importance of asking about glaucoma. Stat Med 1998;289(1):350-355. http://dx.doi.org/10.1000/hgjr.182
- *Book references:* Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975:96-101.
- *Chapter/section in a book:* Weinstein L, Swartz MN. Pathogenic Properties of Invading Microorganisms. In: Sodeman WA, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974:457-472.

- *Internet references:* World Health Organization. The World Health Report 2002 - Reducing Risks, Promoting Healthy Life. Geneva: WHO, 2002. http://www.who.int/whr/2002 (accessed 16 January 2010).
- Legal references

    - Government Gazettes:

    National Department of Health, South Africa. National Policy for Health Act, 1990 (Act No. 116 of 1990). Free primary health care services. Government Gazette No. 17507:1514. 1996.

    In this example, 17507 is the Gazette Number. This is followed by :1514 - this is the notice number in this Gazette.

    - Provincial Gazettes:

    Gauteng Province, South Africa; Department of Agriculture, Conservation, Environment and Land Affairs. Publication of the Gauteng health care waste management draft regulations. Gauteng Provincial Gazette No. 373:3003, 2003.

    - Acts:

    South Africa. National Health Act No. 61 of 2003.

    - Regulations to an Act:

    South Africa. National Health Act of 2003. Regulations: Rendering of clinical forensic medicine services. Government Gazette No. 35099, 2012. (Published under Government Notice R176).

    - Bills:

    South Africa. Traditional Health Practitioners Bill, No. B66B-2003, 2006.

    - Green/white papers:

    South Africa. Department of Health Green Paper: National Health Insurance in South Africa. 2011.

    - Case law:

    Rex v Jopp and Another 1949 (4) SA 11 (N)

    Rex v Jopp and Another: Name of the parties concerned

    1949: Date of decision (or when the case was heard)

    (4): Volume number

    SA: SA Law Reports

    11: Page or section number

    (N): In this case Natal - where the case was heard. Similarly, (C) woud indicate Cape, (G) Gauteng, and so on.

    NOTE: no. after the v

- *Other references (e.g. reports) should follow the same format:* Author(s). Title. Publisher place: Publisher name, year; pages.

- Cited manuscripts that have been accepted but not yet published can be included as references followed by '(in press)'.
- Unpublished observations and personal communications in the text must **not** appear in the reference list. The full name of the source person must be provided for personal communications e.g. (Prof. Michael Jones, personal communication)'.

Ethics Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Apamu Jacques Mapele

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M190725

NAME: (Principal Investigator)	Dr Apamu Jacques Mapele
DEPARTMENT:	Paediatrics and Child Health Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	AN AUDIT OF CULTURE-PROVEN NEONATAL SEPSIS AT TERTIARY HOSPITAL IN SOUTH AFRICA
DATE CONSIDERED:	26/07/2019
DECISION:	Approved Uncondtionally
CONDITIONS:	
SUPERVISOR:	Prof Daynia Ballot
APPROVED BY:	Doctor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	02/08/2019

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 Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. 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 resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **July** and will therefore be due in the month of **July** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

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