EPIDEMIOLOGY OF ACINETOBACTER SEPSIS IN INFANTS ADMITTED TO A NEONATAL UNIT

Reenu Thomas

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfillment of the requirements for the degree of Master in Medicine.

Johannesburg 2015

DECLARATION

I, Reenu Thomas, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Paediatrics, in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University. Any information used in this research report has been obtained by me, Reenu Thomas, while employed by the Chris Hani Baragwanath Academic Hospital and the University of the Witwatersrand.

Signed: _____

On this: 18th day of: January, 2016

DEDICATION

Dedicated to my two beautiful children, Kishan Hitesh Diar and Aashna Diar

PUBLICATION AND PRESENTATIONS

Publications

- Propose to submit to the Journal of Perinatology (see guidelines for submission in Appendix D)

Conference presentations

- United South African Neonatal Association (USANA) Conference, 18th - 20th September 2015, Southern Sun Maharani-Elangeni Hotel, Durban, Kwa-Zulu Natal

ABSTRACT

Background: Acinetobacter baumannii (A. baumannii) is emerging as one of the pathogens causing sepsis in neonates. Prevalence, antibiotic susceptibilities and case-fatality rate (CFR) of A. baumannii in the neonatal units are not well known.

Objective: To determine the prevalence, antibiotic susceptibility patterns and CFR of A. baumannii infection in neonates.

Methods: Medical records of neonates admitted to Chris Hani Baragwanath Academic Hospital from 1st October 2007 to 31st October 2011 with a positive blood or cerebrospinal fluid culture due to A. baumannii were reviewed for demographic characteristics, clinical presentation, laboratory findings, antibiotic susceptibility and outcome.

Results: There were 399 isolates of A. baumannii, with a prevalence of 4.3/1000 live births or 2/ 1000 patient-days, and accounting for 13% of all bacterial and fungal isolates. Antimicrobial susceptibility results were available for 379 isolates and only 155 medical records could be retrieved for analysis. The mean gestational age and birth weight of infected neonates was 30 weeks and 1400 grams respectively. Thirty seven (24%) were isolated from neonates with early onset sepsis and 118 (76%) from those with late onset sepsis. Sixty four percent of isolates were susceptible to Cephalosporins, 21% to Aminoglycosides and 17% were multi-drug resistant (MDR) isolates. The CFR was 32%. Factors associated with mortality were presence of a central venous catheter prior to onset of sepsis (49% vs 31%, p=0.03); need for ventilatory support (62% vs 36%, p=0.005) and inotropic support (57% vs 17%, p<0.001).

Conclusions: A. baumannii is a common pathogen causing sepsis in neonates, with 17% of them being MDR. It is associated with high CFR. These findings highlight the need for strict enforcement of infection control and antibiotic stewardship practices.

ACKNOWLEDGEMENTS

I wish to acknowledge the following individuals who have made this dissertation a reality.

- My supervisor, Professor Sithembiso Velaphi for his kindness, commitment, patience and passion for research.
- Drs' Jeanette Wadula and Sharona Seetharam and the microbiology department at CHBAH for allowing me access to their microbiology records.
- My parents, Mr Thomas Mathew and Mrs Susan Thomas, for their unconditional support and confidence in me.
- My husband, Hitesh Amrat Diar, for being my strength and role model.
- My children, Kishan Hitesh Diar and Aashna Diar, for their understanding and love and for being my inspiration.

TABLE OF CONTENTS

LIST OF TABLES

MAIN RESEARCH REPORT

1.0 INTRODUCTION

Neonatal mortality rates in developing countries have been reported to be as high as 40-50 per 1000 live births.[1] Of these, infections are one of the major contributors, accounting for up to 56% of hospital deaths.[2] In South Africa perinatal conditions account for up to 31% of infant mortality rates, of which 7% have been attributed to infections.[3] Infections in neonates can either be acquired from the mother or from the hospital. Organisms causing infections within the first 72 hours of life are often acquired from the mother. Neonatal nosocomial infections refer to infections in the newborn that are hospital-acquired and usually occurring after the first 72 hours of life.[4]Premature neonates are at high risk of infection due to their underdeveloped innate immunity, fragile skin and lack of protective maternal antibodies. The increasing survival rate of these premature babies in neonatal units has led to an increase in duration of hospital stay, rendering them more susceptible to nosocomial infections. The National Institutes of Child Health and Human Development (NICHD) Neonatal Research Network reported on a cohort of 6215 very-low-birth-weight infants (401-1500 grams) [5], that 21% of these infants developed late-onset or nosocomial neonatal sepsis, of which 18% were caused by gram-negative bacteria. Infection was inversely proportional to birth weight and gestational age. Infants who developed late-onset neonatal sepsis were found to have prolonged hospital stay. Overall mortality was reported to be as high as 18%, especially in babies infected with gram-negative bacteria. Other studies have quoted the incidence of nosocomial infections ranging from 7%-24%.[5, 6, 7] In a recent large cohort study of babies admitted to a neonatal intensive care unit, 14.2% developed late-onset neonatal sepsis, with an overall mortality of 12.6%. Gram negative sepsis accounted for 10.4% of the sepsis attributable mortality.[8] Nosocomial

infections have also been shown to increase the risk of neuro-developmental and growth impairment.[9, 10]

Among the gram-negative bacteria causing infections, Acinetobacter baumannii has recently emerged as a leading nosocomial pathogen responsible for numerous hospital outbreaks over the past decade.[11-13] In a recent cohort, gram negative bacilli accounted for 32.6% of the late-onset neonatal infections, of which Acinetobacter baumannii accounted for 4.2%.[8] Acinetobacter baumannii are aerobic gram-negative cocco-bacilli, belonging to the larger family of Acinetobacter species.[14] These organisms have been recovered from soil, water, animals and humans. They are normal inhabitants of human skin, which in turn could be the major source of severe infections.[15] In addition, various studies have shown the ability of Acinetobacter baumannii to survive in the hospital environment for prolonged periods, potentially playing a role in transmission of the organism during outbreaks. Sources have been shown to include reusable medical equipment, mattresses and gloves.[16-19]

The main concern with Acinetobacter baumanni is its ability to accumulate mechanisms of antimicrobial resistance rapidly, leading to multi-drug resistance. Acinetobacter baumanni has been shown to have acquired resistance to various classes of antimicrobials including Penicillin's, Aminoglycosides, first, second and third generation Cephalosporins and more recently, Fluoroquinolones and Carbapenems. [12, 13, 20, 21, 22] Antibiotics that Acinetobacter baumannii has not developed resistance against so far are the Polymyxins (Colistimethate sodium/ Colistin). The most common mechanism of resistance to β-lactam antibiotics is the ability of the organism to produce β-lactamases. Other mechanisms of resistance include chromosomal mutations as well as loss of specific outer membrane proteins

responsible for influx of antibiotics into the cell.[14] The main sites of infection include the respiratory tract, urinary tract, bloodstream, wounds and burns.[21] Risk factors identified for the development of Acinetobacter baumannii infection include prematurity, very-low-birth-weight, age < 7 days, mechanical ventilation, use of central venous catheters, as well as prior broad spectrum antibiotic use.[12, 13] The mortality rates associated with Acinetobacter baumannii infections in neonates range from 14% to 80%. [12, 13, 23] A South African study reported a mortality rate of 22% .[19]

In the neonatal unit at Chris Hani Baragwanath Academic Hospital (CHBAH) we have seen a high number of infants with positive cultures from normally sterile sites with isolates of Acinetobacter baumannii over the last few years. There also have been outbreaks due to this organism. The mortality due to Acinetobacter baumannii seems to vary during the different outbreaks and reasons for this are not clear. Possible reasons for this variation in mortality might include changes in antibiotic susceptibility, severity of illness or virulence of the organism and underlying problems in the infected patients. Therefore the objectives of this study were to determine characteristics of patients with positive culture from sterile sites due to Acinetobacter baumannii, its antibiotic susceptibilities, case fatality rates and factors associated with mortality. The approval to conduct this study was obtained from the University of Witwatersrand Human Research Ethics Committee, and CHBAH.

2.0 METHODS

2.1 Study design: This is a retrospective descriptive study.

2.2 Study population: The study sample included all babies admitted in the neonatal unit at CHBAH with culture confirmed Acinetobacter baumannii, from blood and/ or cerebrospinal fluid, during the study period 1st October 2007 to 31st October 2011.

2.3 Study procedures: Names and hospital numbers of babies who were infected with Acinetobacter baumannii were obtained from the microbiology department register. These were used to retrieve hospital bed-letters from the neonatal department filing room. Data obtained from these bed-letters were entered into a computerized database for analysis. A case of Acinetobacter baumannii sepsis was defined as a neonate with confirmed culture positive with Acinetobacter baumannii from one or more normally sterile sites. Sites that were considered normally sterile were blood and cerebrospinal fluid. Multiple blood cultures yielding the same organism from the same patient within 72 hours of each other was considered to be a single infection. Antibiotic susceptibilities of Acinetobacter baumannii isolates were retrieved from the laboratory database. Acinetobacter baumannii was defined as multi-drug resistant (MDR) when the organism was resistant to all antibiotics except the Polymyxins. Patient's demographics, clinical management, laboratory findings, and antibiotic susceptibilities were compared between those who died and those who survived.

2.3.1 Data Collection: Information collected included prenatal factors such as patient demographics and maternal human immunodeficiency virus (HIV) status. Postnatal factors obtained included birth weight and age of the baby at the onset of Acinetobacter baumannii

sepsis, use of central venous catheters, total parenteral nutrition and antibiotics prior to the onset of sepsis, any surgical/ invasive procedures underwent prior to the onset of sepsis, need for mechanical ventilation, duration of ventilation, need for inotropic support, length of hospital stay as well as death or survival within 7 days of diagnosis of the infection. Clinical signs, laboratory findings namely full blood count, C-reactive protein (CRP) and cerebrospinal fluid chemistry and cell count at the time of diagnosis of sepsis were also collected. Data were captured onto Microsoft® Office Excel® 2010

2.3.2. Data analysis: Statistical analysis was done using Statistica® version 12. Means and standard deviations were used to describe continuous variables with normal distribution; and medians and ranges were used to describe continuous variables that were not normally distributed. Frequencies and proportions were used to describe categorical variables. In comparing the susceptible to multi-drug resistant or survivors to non-survivors, the Student t-test was used when comparing parametric data, while Mann-Whitney U test was used to compare non-parametric data and the Pearson Chi-square test was used to compare categorical variables. Differences between the two groups were considered to be significant when the p-value was less than <0.05.

3.0 RESULTS

Between October 2007 and October 2011, of all patients admitted to the neonatal unit, there were 399 patients with Acinetobacter baumannii isolated from blood and/or CSF, resulting in an overall prevalence of 4.3 per 1000 live births and 2 per 1000 patient days. The prevalence remained relatively constant through the years, at 4.2, 5.0, 4.1, 4.2 and 3.8 per 1000 live births and 1.9, 2.3, 2.0, 2.2 and 1.9 per 1000 patient days for 2007, 2008, 2009, 2010 and 2011 respectively. Acinetobacter baumannii accounted for 13% of the

3005 bacterial and fungal isolates (excluding those considered to be contaminants) identified over this 4 year period. Of the 399 Acinetobacter baumannii isolates, 379 had susceptibility data available for analysis (95%) and only 155 files could be retrieved for analysis of clinical data (39%).

3.1 Baseline characteristics:

Baseline maternal and neonatal characteristics are shown in Table 1. There were an equal number of males and females. Ninety one percent (91%) of the infants were preterm, with a mean gestational age of 30.5 weeks. Of these, the majority (87%) was between 28 and 34 weeks gestational age. The mean birth weight was 1401 grams, with 72% of the infants being very low birth weight. Thirty two percent of the patients were HIV exposed. The median age of onset of sepsis was 6 days. Twenty four percent (24%) of patients presented as early onset neonatal sepsis, that is within the first 3 days of life and the rest presented as late onset sepsis.

Clinical presentation, laboratory findings at onset of infection and management interventions before onset of infection are summarized in Table 2. Thirty three percent of patients had received parenteral nutrition, and 36% had a central line in situ prior to the onset of infection. Nine percent of infants had surgery prior to the onset of infection. The most common clinical presentation at the time of assessment of infection was respiratory distress, followed by apneas, abdominal distension, increased gastric aspirates and hyperglycemia. Although majority of patients had a normal white cell count at the time of the sepsis screen, a high proportion of 29% were leucopenic (white cell count $< 5 \times 10^9$/ L). Seventy three (47%) infants presented with thrombocytopenia (platelet count below 150×10^9/L). Seventy eight (50%) infants had an elevated CRP (>10mg/L). Of the 155

babies, seventeen (11%) developed A. baumannii sepsis whilst on ventilator support. Of the remaining hundred and thirty eight (89%) patients, sixty (43%) required ventilation at the time of sepsis onset. Forty five (29%) of the patients required some form of inotropic support. The mean duration of treatment was 8 days and the mean length of hospital stay was 29 days. The case fatality rate, which was defined as mortality within 7 days of onset of sepsis, attributable to Acinetobacter baumannii infection, was 32%.

Table 1: Baseline characteristics and interventions before sepsis onset in neonates with positive blood or cerebrospinal fluid culture

Variable	**n (%)**
Maternal HIV status	
Positive	49 (32)
Negative	103 (66)
Unknown	3 (2)
Gestational age	
<28 weeks	29 (19)
28-34 weeks	105 (68)
35-37 weeks	6 (4)
>37 weeks	13 (8)
Unknown	2 (1)
Birth weight	
<1000 grams	38 (24)
1000-1499 grams	74 (48)
1500-2499 grams	28 (18)
≥2500 grams	15 (10)
Male sex	83 (54)
Median Apgar score at 1 minute (IQR)*	7 (5-8)
Median Apgar score at 5 minutes (IQR)*	9 (7-10)
Interventions before sepsis onset	
Central venous access	56 (36)
Parenteral nutrition	51 (33)
Surgery	14 (9)
Receiving ventilator support at onset of sepsis	17 (11)

*IQR = Inter-quartile range: 25^{th} – 75^{th} percentile

Table 2. Clinical presentation and laboratory markers

Variable	**n (%)**
Age at onset of sepsis	
Early-onset sepsis (onset ≤72 hours of life)	37 (24)
Late-onset sepsis (onset >72 hours of life)	118 (76)
Clinical presentation	
Respiratory distress	34 (22)
Apnoea	28 (18)
Abdominal distension	26 (17)
Large gastric aspirates	17 (11)
Hyperglycaemia	12 (8)
White cell count ($x10^9$/L)	
<5	45 (29)
5.0 – 25.0	87 (56)
>25	11 (7)
Unknown	12 (8)
Platelet count ($x10^9$/L	
<100	56 (36)
100-150	17 (11)
>150	69 (45)
Unknown	13 (8)
C-reactive protein (mg/L)	
<10	61 (40)
10.0-20.0	14 (9)
>20	64 (41)
Unknown	16 (10)
Normally sterile sites organism cultured from	
Blood only	145 (94)
CSF only	3 (2)
Blood and CSF	7 (4)
Number requiring mechanical ventilation*	60 (43)
Number requiring inotropes	45 (29)
Number died	49 (32)

*****Among babies who were not already on ventilator support (n=138)

3.2 Antibiotic susceptibilities:

Of the 399 isolates with Acinetobacter baumannii, 379 susceptibilities were available for review (Table 3 and 4). Two hundred and fourty two (64%) of the isolates were susceptible to Cephalosporins. Eighty one (21%) were sensitive to Aminoglycosides. Only 4% were sensitive to Carbapenems. Sixty four (17%) were resistant to multiple antibiotics (multi-drug resistant - MDR) except to the Polymyxins namely Colistin. Over the four year period

the susceptibility of Acinetobacter baumannii to Cephalosporins was between 53 and 70%, and that to Aminoglycoside was 16-35% (Table 3). Susceptibility to Piperacillin-Tazobactam (Tazocin) declined over the years. Susceptibility to Ciprofloxacillin and Carbapenems increased over the years. The proportion of isolates that were MDR remained consistently high over the four year period ranging from 13 to 20%. Among those that were susceptible to the Cephalosporins, most were sensitive to Ceftazidime and among those that were susceptible to the Aminoglycosides most were sensitive to Tobramycin (Table 4).

Table 3: Susceptibility of Acinetobacter baumannii to different groups of antibiotics

	2007 N=23	**2008 N=105**	**2009 N=90**	**2010 N=91**	**2011 N=70**	**TOTAL N=379**
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Cephalosporins						
– Sensitive	13 (57)	70 (67)	63 (70)	48 (53)	48 (69)	242 (64)
– Resistant	10 (43)	35 (33)	27 (30)	43 (47)	22 (31)	137 (36)
Aminoglycosides						
– Sensitive	8 (35)	26 (25)	14 (16)	20 (22)	13 (19)	81 (21)
– Resistant	15 (65)	79 (75)	76 (84)	71 (78)	57 (81)	298 (79)
Carbapenems						
– Sensitive	0 (0)	10 (10)	1 (1)	0 (0)	4 (6)	15 (4)
– Resistant	23 (100)	95 (90)	89 (99)	91 (100)	66 (94)	364 (96)
Piperacillin-Tazobactam						
– Sensitive	1 (4)	17 (16)	3 (3)	2 (2)	1 (1)	24 (6)
– Resistant	22 (96)	88 (84)	87 (97)	89 (98)	69 (99)	355 (94)
Ciprofloxacillin						
– Sensitive	2 (9)	3 (3)	1 (1)	10 (11)	11 (16)	27 (7)
– Resistant	21 (91)	102 (97)	89 (99)	81 (89)	59 (84)	352 (93)
MDR	3 (13)	18 (17)	14 (16)	18 (20)	11 (16)	64 (17)
Colistin						
– Sensitive	23 (100)	105 (100)	90 (100)	91 (100)	70 (100)	379 (100)
– Resistant	0	0	0	0	0	0

MDR – multi-drug resistant

Table 4: Susceptibility of Acinetobacter baumannii isolates to specific antibiotics.

	2007 N=23	**2008 N=105**	**2009 N=90**	**2010 N=91**	**2011 N=70**	**TOTAL N=379**
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Cephalosporins						
– Ceftazidime	11 (48)	63 (60)	49 (54)	47 (52)	46 (66)	216 (57)
– Cefepime	13 (57)	63 (60)	57 (63)	14 (15)	13 ((19)	160 (42)
Aminoglycosides						
– Tobramycin	8 (35)	20 (19)	12 (13)	16 (18)	10 (14)	66 (17)
– Amikacin	0 (0)	7 (67)	4 (4)	4 (4)	4 (6)	19 (5)
– Gentamicin	1 (4)	1 (1)	0 (0)	0 (0)	0 (0)	2 (1)
Carbapenems						
– Meropenem	0 (0)	6 (6)	1 (1)	0 (0)	4 (6)	11 (3)
– Imipenem	0 (0)	5 (5)	0 (0)	0 (0)	1 (1)	6 (2)

3.3 Characteristics of babies infected with susceptible isolates compared to those infected with multi-drug resistant isolates.

Among the 155 files retrieved for analysis, susceptibility results were available for 153 isolates. One hundred and thirty two patients (86%) were infected with susceptible isolates and 21 were infected with MDR isolates (14%). There were no significant differences in baseline characteristics between the 2 groups. Hyperglycemia was seen more frequently in the MDR group compared to the susceptible group (25% vs 5%; p=0.003). There were no cases of Acinetobacter baumannii meningitis in the MDR group, compared with 7% in the susceptible group (p=0.048).

Table 5. Comparing baseline characteristics and interventions before sepsis onset between susceptible and MDR isolates

Variable	**Susceptible N = 132 n (%)**	**MDR N = 21 n (%)**	**p-value**
Maternal HIV status*			0.52
Positive	40 (31)	8 (38)	
Negative	89 (69)	13 (62)	
Gestational age*			0.45
<28 weeks	26 (20)	3 (14)	
28-34 weeks	88 (68)	15 (71)	
35-37 weeks	4 (3)	2 (10)	
>37 weeks	12 (9)	1 (5)	
Birth weight			0.95
<1000 grams	32 (24)	5 (24)	
1000-1499 grams	62 (47)	11 (52)	
1500-2499 grams	25 (19)	3 (14)	
≥2500 grams	13 (10)	2 (10)	
Male sex	70 (53)	11 (52)	0.96
Median Apgar score at 1 minute (IQR)	7 (5-8)	5 (4-8)	0.05
Median Apgar score at 5 minutes (IQR)	9 (4-10)	8 (5-10)	0.77
Interventions before sepsis onset*			
Central venous access	44 (34)	10 (48)	0.22
Parenteral nutrition	45 (35)	6 (29)	0.59
Surgery	13 (10)	1 (5)	0.45

*Maternal HIV status and gestational age was unknown in 3 and 2 patients respectively; interventions before sepsis onset: central venous access - 2 for susceptible, parenteral nutrition - 2 for susceptible, surgery - 1 for susceptible

3.4 Outcome

Of the 155 cases, outcome data were available for 151 babies. There were 102 survivors and 49 deaths at 7 days after onset of infection. Case fatality rate was 32%. There were no statistically significant differences in case fatality rates between babies with MDR Acinetobacter baumannii (29%) and those with susceptible Acinetobacter baumannii (32%).

Table 6. Comparing clinical characteristics between susceptible and MDR isolates

Variable	**Susceptible N = 132 n (%)**	**MDR N = 21 n (%)**	**p-value**
Age at onset of sepsis			0.22
Early-onset sepsis	28 (21)	7 (33)	
Late-onset sepsis	104 (79)	14 (67)	
Clinical presentation*			
Respiratory distress	27 (21)	6 (30)	0.36
Apnoea	27 (21)	1 (5)	0.09
Abdominal distension	24 (19)	2 (10)	0.35
Large gastric aspirates	16 (12)	1 (5)	0.33
Hyperglycaemia	7 (5)	5 (25)	0.003
White cell count ($x10^9$/L)*			0.27
<5	39 (32)	6 (31)	
5.0 – 25.0	77 (62)	10 (53)	
>25	7 (6)	3 (16)	
Platelet count ($x10^9$/L)*			0.26
<100	52 (43)	4 (22)	
100-150	14 (11)	3 (17)	
>150	57 (46)	11 (61)	
C-reactive protein (mg/L)*			0.99
<10	52 (44)	9 (45)	
10,0-20,0	11 (9)	2 (10)	
>20	55 (47)	9 (45)	
Normally sterile site cultured			0.048
Blood only	122 (93)	21 (100)	
CSF only	7 (5)	0 (0)	
Blood and CSF	3 (2)	0 (0)	
Number requiring mechanical ventilation**	53 (45)	7 (39)	0.65
Number requiring inotropes	40 (30)	4 (19)	0.29
Number died	41 (32)	6 (29)	0.75

*Missing variables- Blood results: white cell count results-11 (9 susceptible, 2 MDR), platelets- 15 (12 susceptible, 3 MDR); c-reactive protein – 15 (14 susceptible, 1MDR); outcome- 4 for susceptible; clinical presentation-4 (3 susceptible, 1 MDR)

**Among babies not already on ventilator support at time of sepsis (n=119 for susceptible; n=18 for MDR)

3.5 Comparison of characteristics between survivors and non-survivors

Baseline characteristics were compared between patients that demised within 7 days of onset of sepsis and those that survived beyond 7 days of onset of sepsis. There were no significant differences in gestational age, birth weight, and HIV exposure. The median 5 minute apgar score was significantly lower in the non-survivors (Table 7). There were significantly more babies with central venous lines prior to the onset of sepsis in the group that died compared to the group that survived (49% vs 31%; p=0.03). There were no statistical significant differences in white cell and platelet counts between survivors and non-survivors (Table 8). There was no significant difference in mortality between babies who developed sepsis while on ventilator support compared to the rest of the babies (41% vs 31%; p=0.41). In the group of babies who were not on a ventilator at the time of sepsis onset, a greater number among the babies who died required ventilator support (62% vs 36%, p=0.005) compared to those who survived. Among the babies who died a greater number required inotropic support (57% vs 17%, p<0.001) compared to those who survived.

Table 7. Comparing baseline characteristics and interventions before sepsis onset between survivors and non-survivors

Variable	**Survivors N = 102 n (%)**	**Non-survivors N = 49 n (%)**	**p-value**
Maternal HIV status*			0.48
Positive	31 (30)	17 (36)	
Negative	71 (70)	30 (64)	
Gestational age*			0.45
<28 weeks	17 (17)	11 (23)	
28-34 weeks	69 (68)	34 (71)	
35-37 weeks	5 (5)	1 (2)	
>37 weeks	10 (10)	2 (4)	
Birth weight			0.06
<1000 grams	20 (20)	18 (37)	
1000-1499 grams	49 (48)	23 (47)	
1500-2499 grams	21 (21)	6 (12)	
≥2500 grams	12 (12)	2 (4)	
Male sex	55 (54)	28 (57)	0.71
Median Apgar score at 1 minute (IQR)	7 (5-8)	6 (5-8)	0.19
Median Apgar score at 5 minutes (IQR)	9 (8-10)	8 (7-9)	0.02
Interventions before sepsis onset*			
Central venous access	31 (31)	24 (49)	0.03
Parenteral nutrition	32 (32)	16 (33)	0.9
Surgery	10 (10)	3 (6)	0.45

*Missing variables- 2 for maternal HIV status among the non-survivors and 2 for gestational age (1 among the survivors and 1 among the non-survivors); interventions before sepsis onset: central venous access- 1 for survivors, parenteral nutrition- 1 for survivors

Table 8. Comparing clinical characteristics between survivors and non-survivors

Variable	**Survivors N = 90 n (%)**	**Non-survivors N = 49 (%) n (%)**	**p-value**
Age at onset of sepsis			0.69
Early-onset sepsis	24 (24)	13 (27)	
Late-onset sepsis	78 (76)	36 (73)	
Clinical presentation*			
Respiratory distress	25 (26)	8 (16)	0.21
Apnoea	15 (15)	12 (24)	0.18
Abdominal distension	21 (21)	4 (8)	0.04
Large gastric aspirates	13 (13)	3 (6)	0.19
Hyperglycaemia	7 (7)	5 (10)	0.52
White cell count ($x10^9$/L)*			0.4
<5	27 (28)	17 (40)	
5.0 – 25.0	62 (65)	23 (53)	
>25	7 (7)	3 (7)	
Platelet count ($x10^9$/L)*			0.17
<100	33 (35)	22 (50)	
100-150	11 (12)	6 (14)	
>150	50 (53)	16 (36)	
C-reactive protein (mg/L)*			0.14
<10	48 (48)	11 (30)	
10.0-20.0	9 (9)	5 (13)	
>20	42 (42)	21 (57)	
Normally sterile site cultured			0.84
Blood only	96 (94)	45 (92)	
CSF only	2 (2)	1 (2)	
Blood and CSF	4 (4)	3 (6)	
Number requiring mechanical ventilation**	33 (36)	26 (62)	0.005
Number requiring inotropes	17 (17)	28 (57)	<0.001
Multi-drug resistant	15 (15)	6 (13)	0.75

*Missing variables – white cell count- 12 (6 –survivors, 6- non-survivors), platelet count – 13 (8- survivors, 5- non-survivors), c-reactive protein – 15 (survivors-3, non-survivors-12),); clinical presentation – 4 for survivors.

** Among babies not already on ventilator support at time of sepsis (n=92 for survivors; n=42 for non-survivors)

4.0 Discussion

This study reports on some of the epidemiologic features of infections caused by Acinetobacter baumannii in a neonatal unit from a tertiary public government hospital. It reports on the prevalence and proportion of patients infected with Acinetobacter baumannii, clinical characteristics of affected patients, the antimicrobial susceptibility patterns, outcomes, and comparison of patient characteristics between those with multi-drug resistant and susceptible Acinetobacter baumannii, and between those who died and those who survived.

The findings in this study are that from October 2007 to October 2011, the prevalence of Acinetobacter baumannii was 4.3 per 1000 live births and 2 per 1000 patient days; Acinetobacter accounted for 13% of bacterial and fungal isolates from normally sterile sites of infants admitted to the neonatal unit; majority of infants infected with this bacteria are born preterm and are very low birth weight infants; more than a third of infants were on parenteral nutrition and had central venous line in situ prior to the time of diagnosis of infection. A significant number of isolates (17%) are multi-drug resistant, only being sensitive to Colistin. While susceptibility has remained the same over this four year period for Cephalosporins and Aminoglycosides, it improved slightly for Carbepenems. It has a high case fatality rate. There were no significant statistically differences between infants infected with susceptible and MDR pathogens in demographic characteristics, laboratory findings and mortality, except that more babies in the MDR group presented with hyperglycemia at onset of sepsis and no babies in this group had CSF involvement. In comparing the survivors and non-survivors the factors associated with mortality were prior use of a central venous line, need for mechanical ventilation and need for inotropes.

This prevalence of Acinetobacter baumannii infection among bacterial and fungal isolates is high compared to the reported prevalence in other neonatal intensive care units from previous studies, ranging from 0.2 – 14.1%.[8, 24, 25, 26] However, the prevalence is lower than the reported prevalence of 21% in a recent South African study.[27] In addition, the incidence of 2 cases per 1000 patient days that we have reported is higher than that of 0.5 per 1000 patient days reported in a recent study.[28] Preterm and very low birth weight infants were mostly affected, in keeping with previous studies looking at associated risk factors.[12, 13, 26] Use of total parenteral nutrition and central venous lines prior to the onset of sepsis was high, at 33% and 36% respectively. The use of central venous catheters has been shown in previous studies to be associated with an increased risk of Acinetobacter baumannii infection.[12, 13, 26] Although 76% of infections were acquired after 3 days of life, it is of concern that 24% of the time, the infection was acquired before 72 hours suggesting maternal acquisition. This suggests the high virulence of the organism in a very vulnerable host population. In a retrospective study conducted in India, Acinetobacter baumannii accounted for 14.4% of early onset neonatal sepsis.[29] In this study, the most common affected system at the onset of infection was the respiratory system (respiratory distress and apneas), followed by gastrointestinal tract (abdominal distension and aspirates). Of note, is that a high proportion of the patients required mechanical ventilation (43%) and inotropic support (29%) suggesting severity of sepsis associated with this organism. Laboratory markers of infection that were suggestive of sepsis in these patients included a high mean CRP, leukopenia and thrombocytopenia.

The degree of multi-drug resistance of the Acinetobacter baumannii isolates is very concerning, with 17% of isolates being only sensitive to Colimycin. The majority of isolates were sensitive to Cephalosporins or Aminoglycosides. Carbapenem resistance was

high. Resistance patterns remained mostly unchanged over the years. Most of the isolates are still susceptible to Cephalosporins compared to other groups of antimicrobials. Carbapenem susceptibility improved slightly. Piperacillin-Tazobactam susceptibility has declined over the years. The increase in susceptibility to Ciprofloxacillin is of concern as this may imply increasing resistance to other agents. The rates of multi-drug resistance remained consistently high over the years. High rates of multi-drug resistance have been shown in many studies. [11, 12, 13, 14, 22] A recent South African study has also demonstrated a high rate of multi-drug resistance among Acinetobacter baumannii isolates.[27] These findings highlight the need for proper infection control and antibiotic stewardship practices. Infection control measures should focus on hand hygiene and adherence to other infection prevention and control (IPC) guidelines. Enforcement of continuing antibiotic stewardship practices will prevent selection of resistant strains of Acinetobacter baumannii, and hence reduce the prevalence of multi-drug resistance. These practices include early discontinuation of empiric antibiotic therapy and appropriate use and duration of antibiotics in blood culture confirmed infections. Regular IPC audits should be carried out in the unit, feedback should be given to the unit and measures to improve infection control and antibiotic stewardship practices should be implemented accordingly.

Surprisingly, there were no significant differences between the baseline characteristics and mortality of patients with multi-drug resistant and susceptible Acinetobacter baumannii. This is not in keeping with other studies which have shown a strong association between mortality and multi-drug resistant Acinetobacter baumannii. [12, 26]

Mortality attributable to Acinetobacter baumannii sepsis was high, at 32%, higher than the reported mortality of 22% from a previous South African study. [19] The reported mortality

rates from other studies range from 14% - 80%. [12, 13, 23, 26] Comparing baseline characteristics of survivors to non-survivors, not surprisingly, the need for mechanical ventilation and inotropic support was significantly higher in babies that died, suggesting that this was a sicker group of infants who in most instances required ventilator and inotropic support, most likely secondary to septic shock, prior to their demise. But this also highlights the need for ventilator and inotropic support as a poor prognostic sign for favourable outcome. In addition, more babies who died had a central venous line placement compared to babies that survived. The presence of central venous lines has been shown to increase the risk of Acinetobacter infection, but as far as we know, has not been shown to be associated with increased mortality.

This study has several limitations. This was a retrospective record review. Although susceptibility patterns could be retrieved on majority of the cases (95%), only 39% of the hospital files could be retrieved for analysis. Of these, outcome data were not available for 3% of cases. Although the sample size is small, to our knowledge, this study represents one of the largest number of studied patients with Acinetobacter baumannii sepsis. In view of the retrospective nature of the study, it is possible that the prevalence of Acinetobacter baumannii infection in the unit is not a true reflection of the actual prevalence as adequate blood cultures may not have been taken at the onset of suspected sepsis, and that could contribute to a lower yield.

In conclusion, the prevalence and proportion of infections due to Acinetobacter baumannii in the unit is high. Of major concern is the high rate of multi-drug resistance as well as case fatality. Our findings highlight the need for strict enforcement of infection control and antibiotic stewardship practices. Factors that contribute to breech of infection control

practices in developing countries, such as overcrowding of nurseries and staff shortages need to be urgently attended to. This study also highlights the need for continuous surveillance programs as well as the need for ongoing research, preferably prospective in nature, on nosocomial infections, resistance patterns as well as interventions aimed at improving infections in neonatal ICUs especially in developing countries.

5.0 References

1. Hyder AA, Wali SA, McGuckin J. The burden of disease from neonatal mortality: a review of South Asia and Sub-Saharan Africa. BJOG. 2003 ; 110: 894-901.
2. Stoll BJ. Neonatal infections: A global perspective. In: Remington JS, Klein JO, eds. Infectious diseases of the fetus and newborn infant. 6th edition. Philadelphia: WB Saunders company, 2006, Chapter 2
3. Nannan N, Dorrington RE, Laubscher R, Zinyakatira N, Prinsloo M, Darikwa T, et al. Under 5 Mortality statistics in South Africa. Shedding some light on the trends and causes 1997-2001. Cape Town: South African Medical Reasearch Council, 2012
4. Baltimore RS. Neonatal Nosocomial Infections. Semin Perinatol. 1998; 22: 25-32.
5. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Late onset sepsis in very low birth weight neonates: The experience of the NICHD Neonatal Research Network. Pediatrics. 2002; 110: 285-91.
6. Aly H, Herson V, Duncan A, Herr J, Bender J, Patel K, El-Mohandes AA. Is bloodstream infection preventable among premature infants? A tale of two cities. Pediatrics. 2005; 115: 1513-8.
7. Aziz K, McMillan DD, Andrews W, Pendray M, Qui Z, Karuri S, et al. Variations in rates of nosocomial infection among Canadian Neonatal Intensive Care Units may be practice related.BMCPediatrics.2005; 5 . Available from: http://www.biomedcentral.com/1471-2431/5/22
8. Tsai MH, Hsu JF, Chu SM, Lien R, Huang HR, Chiang MC, et al. Incidence, clinical characteristics and risk factors for adverse outcome in neonates with late onset neonatal sepsis. Pediatr Infect Dis J. 2014 30; 33 : e7 – e 13.

9. Stoll BJ, Hansen NI, Adams-Chapman I, Fanaroff AA, Hintz SR, Vohr B, et al. Neurodevelopmental and growth impairment among extremely low birth weight infants with neonatal infection. JAMA. 2004 ; 292 : 2357-65.
10. Mitha A, Foix-L'Helias L, Arnaud C, Marret S, Vieux R, Aujard Y, et al. Neonatal Infection and 5 year neurodevelopmental outcomes of very preterm infants. Pediatrics. 2013; 132: e372-80.
11. Simmonds A, Munoz J, Aquero-Rosenfeld M, Carbonaro C, Montecalvo M, Clones B, et al. Outbreak of Acinetobacter infection in extremely low birth weight neonates. Pediatr Infect Dis J. 2009; 28: 210-14.
12. Al Jarousha AM, El Jadba AH, Al Afifi AS, El Qouqa IA. Nosocomial multi-drug resistant Acinetobacter baumannii in the Neonatal Intensive Care Unit in Gaza city, Palestine. Int J Infect Dis. 2009; 13: 623-8.
13. Touati A, Achour W, Cherif A, Hmida HB, Afif FB, Jabnoun S, et al. Outbreak of Acinetobacter baumannii in a Neonatal Intensive Care Unit: Antimicrobial and Genotype Analysis. Ann Epidemiol. 2009; 19: 372-8.
14. Hanlon GW. The emergence of multidrug resistant Acinetobacter baumannii species: A major concern in the hospital setting. Lett Appl Microbiol. 2005; 41: 375-8.
15. Fournier PE, Richet H. The epidemiology and control of Acinetobacter baumannii in health care facilities. Clin Infect Dis. 2006; 42: 692-9.
16. Wendt C, Dietze B, Dietz E, Ruden H. Survival of Acinetobacter baumannii on dry surfaces. J Clin Microbiol. 1997; 35: 1394-7.
17. Catalano M, Quelle LS, Jeric PE, Di Martino A, Maimone SM. Survival of Acinetobacter baumannii on bed rails during an outbreak and during sporadic cases. J Hosp Infect. 1999 ; 42: 27-35

18. Denton M, Wilcox MH, Parnell P, Green D, Keer V, Hawkey PM, et al. Role of environmental cleaning in controlling an outbreak of Acinetobacter baumannii in a neurological intensive care unit. J Hosp Infect. 2004; 56: 106-10.
19. Pillay T, Pillay DG, Adhikary M, Pillay A, Sturm AW. An outbreak of neonatal infection with Acinetobacter linked to contaminated suction catheters. J Hosp Infect. 1999 ; 43: 299-304.
20. Abbo A, Navon-Venezia S, Hammer-Muntz O, Krichali T, Siegman-Igra Y, Carmeli Y. Multidrug resistant Acinetobacter baumannii. Emerg Infect Dis. 2005; 11: 22-9.
21. Karlowsky JA, Draghi DC, Jones ME, Thornsberry C, Friedland IR, Sahm DF. Surveillance for antimicrobial susceptibility among clinical isolates of Pseudomonas Aureginosa and Acinetobacter baumannii from hospitalized patients in the United States, 1998-2001. Antimicrob Agents Chemother. 2003; 47: 1681-8.
22. Turton JF, Kaufmann ME, Warner M, Coelho J, Dijkshoorn L, van der Reijden T, et al. A prevalent multi-resistant clone of Acinetobacter baumannii in South East England. J Hosp Infect. 2004; 58: 170-9.
23. Von Dolinger De Brito D, Oliveira EJ, Abdallah VO, da Costa Darini AL, Filho PP. An outbreak of Acinetobacter baumannii septicemia in a Neonatal Intensive Care Unit of a University Hospital in Brazil. Braz J Infect Dis. 2005; 9: 301- 9.
24. Bas AY, Demirel N, Zenciroglu A, Gol N, Tanir G. Nosocomial bloodstream infections in a neonatal intensive care unit in Ankara, turkey. Turkish J Pediatr, 2010; 52: 464-70.
25. Zakariya BP, Bhat V, Harish BN, Arun Babu T, Joseph NM. Neonatal sepsis in a tertiary hospital in South India: Bacteriological profile and antibiotic sensitivity pattern. Indian J Pediatrics, 2011; 78 : 413-7

26. Thatrimontrichai A, Apisarnthanarak A, Chanvitan P, Janjindamai W, Dissaneevate S, Maneelil G. Risk factors and Outcomes of Carbapenem-resistant Acinetobacter baumannii Bacteremia in Neonatal Intensive Care Unit: A Case-case control study. Pediatric Inf Dis J, 2013; 32: 140-5.
27. Morkel G, Bekker A, Marais BJ, Kirsten G, Van Wyk J, Dramowski A. Bloodstream infections and antimicrobial resistance patterns in a South African neonatal intensive care unit. Paediatr Int Child Health, 2014; 34: 108-114.
28. Kumar A, Randhawa VS, Nirupam N, Rai Y, Saili A. Risk factors for carbapenem resistant Acinetobacter baumannii blood stream infections in a neonatal intensive care unit, Delhi, India. J Infect Dev Ctries, 2014; 8; 1049-1054.
29. Bhat YR, Lewis LE, Vandana KE. Bacterial isolates of early onset neonatal sepsis and their antibiotic susceptibility pattern between 1998 and 2004: an audit from a center in India. Ital J Pediatr, 2011; 37: 32.

6.0 APPENDICES

6.1 APPENDIX A:

PROTOCOL

Background

Neonatal Mortality rates in developing countries has been reported to be as high as 40-50 per 1000 live births.[1] Of these, infections are one of the major killers, accounting for up to 56% of hospital deaths.[2] In South Africa perinatal conditions account for up to 31% of infant mortality rates, of which 7% have been attributed to infections.[3] Infections in neonates can either be acquired from the mother or from the hospital. Organisms causing infections within the first 72 hours of life is often acquired from the mother. Neonatal nosocomial infections refer to infections in the newborn that are hospital-acquired and usually occurring after the first 72 hours of life.[4] Premature neonates are at high risk of infection due to their underdeveloped innate immunity, fragile skin and lack of protective maternal antibodies. The increasing survival rate of these premature babies in neonatal units has led to an increase in duration of hospital stay, rendering them more susceptible to nosocomial infections.

The National Institutes of Child Health and Human Development (NICHD) Neonatal Research Network reported on a cohort of 6215 very-low-birth-weight infants (401-1500 grams).[5] Twenty-one percent (21%) of these infants developed late-onset neonatal sepsis, of which 18% were caused by gram-negative organisms. Infection was inversely proportional to birth weight and gestational age. Infants who developed late-onset neonatal sepsis were found to have prolonged hospital stay. Overall mortality was reported to be as high as 18%, especially in babies infected with gram-negative organisms. Other studies have quoted the incidence of nosocomial infections ranging from 7%-24%.[5, 6, 7] In a recent

large cohort study of babies admitted to a neonatal intensive care unit, 14.2% developed late-onset neonatal sepsis, with an overall mortality of 12.6%. Gram negative sepsis accounted for 10.4% of the sepsis attributable mortality.[8] Nosocomial infections has been also shown to increase the risk of neuro-developmental and growth impairment.[9, 10] Among the gram-negative bacterial organisms causing infections, Acinetobacter baumannii has recently emerged as a leading nosocomial pathogen responsible for numerous hospital outbreaks over the past decade.[11, 12, 13] In a recent cohort, gram negative bacilli accounted for 32.6% of the late-onset neonatal infections, of which Acinetobacter baumannii accounted for 4.2%.[8] Acinetobacter baumannii are aerobic gram-negative cocco-bacilli, belonging to the larger family of Acinetobacter species.[19] These organisms have been recovered from soil, water, animals and humans. They are normal inhabitants of human skin, which in turn could be the major source of severe infections.[14] In addition, various studies have shown the ability of Acinetobacter baumannii to survive in the hospital environment for prolonged periods, potentially playing a role in transmission of the organism during outbreaks. Sources have been shown to include reusable medical equipment, mattresses and gloves.[15, 16, 17, 18]

The main concern with Acinetobacter baumannii is its ability to accumulate mechanisms of antimicrobial resistance rapidly, leading to multi-drug resistance. Acinetobacter baumannii has been shown to have acquired resistance to various classes of antimicrobials including Penicillin's, Aminoglycosides, first, second and third generation Cephalosporin's and more recently, Fluoroquinolones and Carbapenems. The Polymyxins (Colistimethate sodium/ Colistin) are the only agents with the overall highest susceptibility rates.[12, 13, 20, 21, 22] The most common mechanism of resistance to β-lactam antibiotics is the ability of the organism to produce β-lactamases. Other mechanisms of resistance include chromosomal

mutations as well as loss of specific outer membrane proteins responsible for influx of antibiotics into the cell.[19]

The main sites of infection include the respiratory tract, urinary tract, bloodstream, wounds and burns.[21] Risk factors identified for the development of Acinetobacter baumannii infection include prematurity, very-low-birth-weight, age < 7 days, prolonged hospital stay > 7 days, mechanical ventilation, use of central venous catheters, as well as prior broad spectrum antibiotic use.[12, 13] The mortality rates associated with Acinetobacter baumannii infections in neonates ranges from 14%-80%. [12, 13, 23] A South African study reported a mortality of 22% .[18]

In our neonatal unit at Chris Hani Baragwanath Academic Hospital (CHBAH), limitations in resources, overcrowding and the lack of isolation facilities leading to nosocomial infections are a major problem. We have seen an increase in numbers of Acinetobacter baumannii as a cause of infection in our neonates over the past years both as an endemic organism as well as a cause of outbreaks. The mortality due to Acinetobacter baumannii seems to vary during the different outbreaks and reasons for this are not clear. Possible reasons for this variation in mortality might include changes in antibiotic susceptibility, severity of illness or virulence of the organism and underlying problems in the infected patients. Therefore we would like to determine characteristics of patients who are infected with this organism, antibiotic susceptibilities of this organism and compare the characteristics of survivors to non-survivors.

Objectives

1. To determine the incidence of sterile sites (blood and cerebrospinal fluid) infections due to Acinetobacter baumannii infection in infants admitted to CHBAH neonatal unit
2. To determine antibiotic susceptibilities of Acinetobacter baumannii isolated from normally sterile sites.
3. To describe the characteristics of infants infected with Acinetobacter baumannii
4. To determine the mortality rates and factors associated with mortality in infants infected with Acinetobacter baumannii.

Justification

1) This study will give us an idea of the burden of Acinetobacter baumannii sepsis in our neonatal unit.
2) By describing characteristics of infants who are more likely to die from Acinetobacter baumannii infection in our population of babies, we can have a higher index of suspicion for poor outcome and therefore encouraging early empiric treatment that includes coverage for Acinetobacter baumannii. Doing this might reduce the mortality rate due to this infection.
3) The results of this study will encourage the enforcement and improvement of infection control measures among staff at CHBAH.
4) This study will provide us with a basis for future randomized control trials and interventional studies involving infection control strategies.

Methods

Study design: This will be a retrospective descriptive study.

Study population: It will include all babies admitted in the neonatal unit at CHBAH from 1st October 2007 to 31st October 2012, who were infected with A. baumannii.

Study procedures and data collection: Names of babies will be obtained from the microbiology department database. Hospital bed-letters of these babies will be retrieved from the department filing room. Data obtained from these bed-letters will be entered into a data collection sheet (see attached) and thereafter into a computerized database and analyzed. Information that will be analyzed will include prenatal factors such as patient demographics and maternal HIV status. Postnatal factors obtained will include age and weight of the baby at the onset of Acinetobacter baumannii sepsis, use of central venous catheters, urinary catheters, antibiotic use prior to the onset of sepsis, any surgical/ invasive procedures, use of total parenteral nutrition, baby's HIV exposure and status if available, need for mechanical ventilation, duration of ventilation, need for inotropic support, length of hospital stay as well as death or survival to discharge. A case of Acinetobacter baumannii sepsis will be defined as a neonate with confirmed culture positive with Acinetobacter baumannii from one or more normally sterile sites. Sterile sites will include blood, and cerebrospinal fluid. Multiple blood cultures yielding the same organism from the same patient within 72 hours of each other will be considered to be a single infection. The antibiotic susceptibilities of Acinetobacter baumannii isolates will be retrieved from the laboratory database. Acinetobacter baumannii will be defined as pan-resistant when the organism is resistant to all antibiotics except the Polymyxins. Clinical presentation at the time of developing the sepsis as well as laboratory markers of infection will be collected for review. Laboratory markers of infection will include white cell counts, including differential counts, platelet counts, C-reactive protein and cerebrospinal fluid (CSF) results. Patient's demographics, clinical presentation, laboratory markers and antibiotic susceptibilities will be compared between those who died and those who survived.

Statistical Analysis

Information collected using the data collection sheet will be analyzed. Means, medians and ranges will be used to describe continuous variables. Frequencies and proportions will be used describe categorical variables. Student t-test and chi-square test will be used to compare continuous and dichotomous variables, respectively between survivors and non-survivors.

Ethics

The approval for conducting this study will been obtained from the University of Witwatersrand Human Research Ethics Committee (HREC), the Hospital Superintendent and the Head of Department of Paediatrics at Chris Hani Baragwanath Academic Hospital. Though names of patients will be used when retrieving the charts, no names of patients or identifiers will be used as part of the data to be collected. All patients will be given study numbers in order to maintain confidentiality.

Timing

I will begin the study as soon as the proposal has been accepted and intend completing it within 6 months.

Funding

No funding will be required. All necessary expenses for paper and printing will be self-funded.

References

1. Hyder AA, Wali SA, McGuckin J. The burden of disease from neonatal mortality: a review of South Asia and Sub-Saharan Africa. BJOG. 2003 Oct; 110(10): 894-901.
2. Stoll BJ. Neonatal infections: A global perspective. In: Remington JS, Klein JO, eds. Infectious diseases of the fetus and newborn infant. 6th edition. Philadelphia: WB Saunders company, 2006, Chapter 2
3. Nannan N, Dorrington RE, Laubscher R, Zinyakatira N, Prinsloo M, Darikwa T, et al. Under 5 Mortality statistics in South Africa. Shedding some light on the trends and causes 1997-2001. Cape Town: South African Medical Reasearch Council, 2012
4. Baltimore RS. Neonatal Nosocomial Infections. Semin Perinatol. 1998 Feb; 22 (1): 25-32.
5. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Late onset sepsis in very low birth weight neonates: The experience of the NICHD Neonatal Research Network. Pediatrics. 2002 Aug; 110 (2): 285-91.
6. Aly H, Herson V, Duncan A, Herr J, Bender J, Patel K, El-Mohandes AA. Is bloodstream infection preventable among premature infants? A tale of two cities. Pediatrics. 2005 Jun; 115 (6): 1513-8.
7. Aziz K, McMillan DD, Andrews W, Pendray M, Qui Z, Karuri S, et al. Variations in rates of nosocomial infection among Canadian Neonatal Intensive Care Units may be practice related. BMC Pediatrics. 2005; 5 (22). Available from: http://www.biomedcentral.com/1471-2431/5/22
8. Tsai MH, Hsu JF, Chu SM, Lien R, Huang HR, Chiang MC, et al. Incidence, clinical characteristics and risk factors for adverse outcome in neonates with late onset neonatal sepsis. Pediatr Infect Dis J. 2013 July 30. DOI: 10.1097/INF.0b013e3182a72ee0

9. Stoll BJ, Hansen NI, Adams-Chapman I, Fanaroff AA, Hintz SR, Vohr B, et al. Neurodevelopmental and growth impairment among extremely low birth weight infants with neonatal infection. JAMA. 2004 Nov 17; 292 (19): 2357-65.
10. Mitha A, Foix-L'Helias L, Arnaud C, Marret S, Vieux R, Aujard Y, et al. Neonatal Infection and 5 year neurodevelopmental outcomes of very preterm infants. Pediatrics. 2013 Aug; 132(2): e372-80.
11. Simmonds A, Munoz J, Aquero-Rosenfeld M, Carbonaro C, Montecalvo M, Clones B, et al. Outbreak of Acinetobacter infection in extremely low birth weight neonates. Pediatr Infect Dis J. 2009 Mar; 28(3): 210-14.
12. Al Jarousha AM, El Jadba AH, Al Afifi AS, El Qouqa IA. Nosocomial multi-drug resistant Acinetobacter baumannii in the Neonatal Intensive Care Unit in Gaza city, Palestine. Int J Infect Dis. 2009 Sep; 13(5): 623-8.
13. Touati A, Achour W, Cherif A, Hmida HB, Afif FB, Jabnoun S, et al. Outbreak of Acinetobacter baumannii in a Neonatal Intensive Care Unit: Antimicrobial and Genotype Analysis. Ann Epidemiol. 2009 Jun; 19(6): 372-8.
14. Fournier PE, Richet H. The epidemiology and control of Acinetobacter baumannii in health care facilities. Clin Infect Dis. 2006 Mar 1; 42(5): 692-9.
15. Wendt C, Dietze B, Dietz E, Ruden H. Survival of Acinetobacter baumannii on dry surfaces. J Clin Microbiol. 1997 Jun; 35(6): 1394-7.
16. Catalano M, Quelle LS, Jeric PE, Di Martino A, Maimone SM. Survival of Acinetobacter baumannii on bed rails during an outbreak and during sporadic cases. J Hosp Infect. 1999 May; 42(1): 27-35
17. Denton M, Wilcox MH, Parnell P, Green D, Keer V, Hawkey PM, et al. Role of environmental cleaning in controlling an outbreak of Acinetobacter baumannii in a neurological intensive care unit. J Hosp Infect. 2004 Feb; 56(2): 106-10.

18. Pillay T, Pillay DG, Adhikary M, Pillay A, Sturm AW. An outbreak of neonatal infection with Acinetobacter linked to contaminated suction catheters. J Hosp Infect. 1999 Dec; 43(4): 299-304.
19. Hanlon GW. The emergence of multidrug resistant Acinetobacter baumannii species: A major concern in the hospital setting. Lett Appl Microbiol. 2005; 41(5): 375-8.
20. Abbo A, Navon-Venezia S, Hammer-Muntz O, Krichali T, Siegman-Igra Y, Carmeli Y. Multidrug resistant Acinetobacter baumannii. Emerg Infect Dis. 2005 Jan; 11(1): 22-9.
21. Karlowsky JA, Draghi DC, Jones ME, Thornsberry C, Friedland IR, Sahm DF. Surveillance for antimicrobial susceptibility among clinical isolates of Pseudomonas Aureginosa and Acinetobacter baumannii from hospitalized patients in the United States, 1998-2001. Antimicrob Agents Chemother. 2003 May; 47(5): 1681-8.
22. Turton JF, Kaufmann ME, Warner M, Coelho J, Dijkshoorn L, van der Reijden T, et al. A pravelant multi-resistant clone of Acinetobacter baumannii in South East England. J Hosp Infect. 2004 Nov; 58(3): 170-9.
23. Von Dolinger De Brito D, Oliveira EJ, Abdallah VO, da Costa Darini AL, Filho PP. An outbreak of Acinetobacter baumannii septicemia in a Neonatal Intensive Care Unit of a University Hospital in Brazil. Braz J Infect Dis. 2005 Aug; 9(4): 301- 9.

6.2 APPENDIX B: ETHICS CLEARANCE CERTIFICATE

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Reenu Thomas

CLEARANCE CERTIFICATE	M10112
PROJECT	Epidemiology of Acinetobacter Sepsis in Infants Admitted to the Neonatal Unit (New title)
INVESTIGATORS	Dr Reenu Thomas.
DEPARTMENT	Department of Paediatrics/Neonatology
DATE CONSIDERED	29/01/2010
M100DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 01/02/2012 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Prof SC Velaphi

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

6.3 APPENDIX C: PLAGIARISM CHECK

Turnitin Originality Report

MMEDTHESISFINAL2.doc by Reenu Thomas
From MMed submission 2015
(8P1Gr6Q2at0C3dybh9vW36k1KFM27938QYKA20NjSvhDWoeS908jGcO1K9xykBzQ1d1fw7nzyHWnjLvlL4hs9fYkH6PQx8ht8og)

Processed on 25-Sep-2015 8:07 AM SAST
ID: 575396818
Word Count: 8755

Similarity Index

20%

Similarity by Source
Internet Sources:
15%

Publications:
16%

Student Papers:
9%

######### END Top Body ##############################

sources:

1
1% match (Internet from 01-Nov-2014)
http://www.myhealthunit.ca/en/partnerandhealthproviderresources/resources/LTCH/BP_Environmental_Cleaning_May_2012.pdf

2
1% match (student papers from 09-Jun-2015)
Submitted to University of Witwatersrand on 2015-06-09

3
1% match (Internet from 15-Jul-2010)
http://www.jlponline.org/article.asp?issn=0974-2727;year=2009;volume=1;issue=2;spage=73;epage=76;aulast=Shete

4
1% match (publications)
G.W. Hanlon. "The emergence of multidrug resistant Acinetobacter species: a major concern in the hospital setting", Letters in Applied Microbiology, 11/2005

5
1% match (publications)
S. Velaphi. "Mortality rate in neonates infected with extended-spectrum

β lactamase-producing Klebsiella species and selective empirical use of meropenem", Annals of Tropical Paediatrics International Child Health, 06/01/2009

6

< 1% match (publications)
Kida, Yoshiko, Shinichiro Ohshimo, Kohei Ota, Tomoko Tamura, Tadatsugu Otani, Kazunobu Une, Takuma Sadamori, Yasumasa Iwasaki, Francesco Bonella, Noboru Hattori, Nobuyuki Hirohashi, Josune Guzman, Ulrich Costabel, Nobuoki Kohno, and Koichi Tanigawa. "KL-6, a Human MUC1 Mucin, as a prognostic marker for diffuse alveolar hemorrhage syndrome", Orphanet Journal of Rare Diseases, 2012.

7

< 1% match (student papers from 16-Nov-2012)
Submitted to The University of Memphis on 2012-11-16

8

< 1% match (Internet from 07-Sep-2009)
http://www.hpa.org.uk/web/HPAwebfile/HPAweb_C/1215762295386

9

< 1% match (Internet from 20-May-2015)
http://jama.jamanetwork.com/article.aspx?articleid=199811

10

< 1% match (Internet from 11-Apr-2014)
http://www.hst.org.za/sites/default/files/SAHR2012_13_lowres_1.pdf

11

< 1% match (student papers from 25-Mar-2012)
Submitted to King Saud University on 2012-03-25

12

< 1% match (Internet from 22-Mar-2010)
http://www.emro.who.int/Publications/EMHJ/1601/article7.htm

13

< 1% match (Internet from 05-Oct-2014)
http://download.pediatriaospedaliera.org/atti-congressuali/Atti_Congresso_Aversa_2009.pdf

14

< 1% match (publications)
A. R. Stark. "Late-Onset Sepsis in Very Low Birth Weight Neonates: The Experience of the NICHD Neonatal Research Network", PEDIATRICS, 08/01/2002

15

< 1% match (publications)
"American Transplant Congress 2007 Executive and Program

Planning Committees and Abstract Review Committees", American Journal of Transplantation, 5/2007

16

< 1% match (student papers from 22-Jul-2015)
Submitted to The University of Manchester on 2015-07-22

17

< 1% match (Internet from 06-Mar-2014)
http://library.nymc.edu/bibliog/Authors08-09.pdf

18

< 1% match (Internet from 13-Jul-2010)
http://www.hpa.org.uk/web/HPAweb&HPAwebStandard/HPAweb_C/1217401231280

19

< 1% match ()
http://www.dhfs.state.wi.us/wish/measures/inf_mort/long_form.html

20

< 1% match (Internet from 12-Mar-2014)
http://cid.oxfordjournals.org/content/42/5/692.full

21

< 1% match (Internet from 21-Jan-2015)
http://robertdebre.aphp.fr/wp-content/blogs.dir/152/files/2014/07/Publications310714.pdf

22

< 1% match (publications)
Fournier, P. E., H. Richet, and R. A. Weinstein. "The Epidemiology and Control of Acinetobacter baumannii in Health Care Facilities", Clinical Infectious Diseases, 2006.

23

< 1% match (publications)
Kitazono, Hidetaka, Dominik Rog, Shellee A. Grim, Nina M. Clark, and Gail E. Reid. "Acinetobacter baumannii infection in solid organ transplant recipients", Clinical Transplantation, 2015.

24

< 1% match (Internet from 23-Jan-2014)
http://wiredspace.wits.ac.za/bitstream/handle/10539/9302/PhD%20Thesis%20OYEDELE%20-%202010.pdf

25

< 1% match (Internet from 23-Sep-2008)
http://www.nature.com/cgi-taf/DynaPage.taf?file=/jp/journal/v25/n1s/full/7211273a.html&filetype=pdf

26

< 1% match (publications)
Villegas, Maria Virginia, and Alan I. Hartstein. "*Acinetobacter* Outbreaks, 1977â€“2000 â€¢â€," Infection Control and Hospital Epidemiology, 2003.

27

< 1% match (publications)
Anah, M U, J J Udo, S O Ochigbo, and L N Abia-Bassey. "Neonatal septicaemia in Calabar, Nigeria", Tropical Doctor, 2008.

28

< 1% match (Internet from 05-May-2015)
http://www.grady.uga.edu/ANNUALSURVEYS/Graduate_Survey/Graduate_2013/Grad_Report_2013_Combined.pdf

29

< 1% match (publications)
Haque, Khalid N, Mohan Pammi, and Mohan Pammi. "Pentoxifylline for treatment of sepsis and necrotizing enterocolitis in neonates", Cochrane Database of Systematic Reviews Reviews, 2011.

30

< 1% match (publications)
Martins, Andreza Francisco and Barth, Afonso Luís. "Acinetobacter multirresistente - um desafio para a saúde pública", Scientia Medica, 2013.

31

< 1% match (publications)
"Preventing Early-onset Group B Streptococcal Sepsis: Strategy Development Using Decision Analysis", PEDIATRICS, 06/01/1999

32

< 1% match (student papers from 26-Nov-2012)
Submitted to Nottingham Trent University on 2012-11-26

33

< 1% match (publications)
Knobloch, Johannes K.M., Martin, Maria, Mattner, Frauke, Messler, Sabine, Ott, Ella, Pfeifer, Yvonne, Schulze-Röbbecke, Roland and von Thomsen, Alexander J.. "Analyse von nosokomialen Ausbrüchen mit multiresistentem Acinetobacter baumannii", Infektionsepidemiologie, 2012.

34

< 1% match (publications)
Robin E. Remsburg, Karen A. Armacost, Cha. "IMPACT OF A RESTORATIVE CARE PROGRAM IN THE NURSING HOME", Educational Gerontology, 4/1/2001

35

< 1% match (student papers from 06-Aug-2015)
Submitted to University of Southampton on 2015-08-06
36
< 1% match (Internet from 15-Nov-2010)
http://www.ijp.iranpath.org/IJPArticles/5_4/Pages%20from%20fil%20asyb%2020-5.pdf
37
< 1% match (Internet from 12-Mar-2015)
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4314163/
38
< 1% match (Internet from 16-Jan-2013)
http://www.ann-clinmicrob.com/content/8/1/18
39

6.4 APPENDIX D: AUTHORS GUIDELINES FOR SUBMISSION TO THE JOURNAL OF PERINATOLOGY

FORMAT OF PAPERS

Article Types Table

Please note that as of 2015, the *Journal of Perinatology* no longer accepts Perinatal/Neonatal Case Presentations or Imaging Casebooks.

Article Type	Description	Word Count
Original Article	Generally, the Journal only considers original research materials that are directly relevant to clinical practice.	Length should not exceed 20 pages including abstract, text, tables, illustrations, and references
State-of-the-Art and Commentaries	State-of-the-Art manuscripts are review articles intended to update readers on important subjects relevant to maternal-fetal and neonatal care. These articles are considered to be complete from the most recent major review in the literature. Authors should contact the Editor before submission of a completed project.	Commentaries are shorter articles tha review a limited topic and/or may exp controversial opinions regarding speci aspects of perinatal and neonatal care practices.
Letters to the Editor	Letters may be brief comments regarding aspects of care or in response to specific published articles in the Journal. Letters are subject to critical review and editorial policy.	
Calendar	Announcements of scheduled meetings, symposia or postgraduate courses may be sent for consideration at least five months in advance of the date of publication desired.	
Other	To submit a manuscript as a **Special Feature** or **State-of-the-Art**, contact the editorial office	

Preparation of Original Articles

Cover letter (must include a Conflict of Interest statement)
Title page (excluding acknowledgements)
Abstract
Introduction
Materials (or patients) and methods
Results
Discussion
Acknowledgements
Conflict of Interest
References
Tables
Figures

Cover letter

The uploaded covering letter must state the material is original research, has not been previously published and has not been submitted for publication elsewhere while under consideration. The covering letter must also contain a Conflict of Interest statement (see Editorial Policy section).

Title page

The title page should bear the title of the paper, the full names of all the authors, highest academic degree obtained, and their affiliations, together with the name, full postal address, telephone and fax numbers and e-mail address of the author to whom correspondence and offprint requests are to be sent (This information is also asked for on the electronic submission form). The title should be brief, informative, of 150 characters or less and should not make a statement or conclusion. The running title should consist of not more than 50 letters and spaces. It should be as brief as possible, convey the essential message of the paper and contain no abbreviations. Authors should disclose the sources of any support for the work, received in the form of grants and/or equipment and drugs.

Abstract

A structured abstract is required for original articles and a standard abstract format is required for other types of articles. An abbreviated unformatted abstract is preferred for State-of-the-Art articles.

The structured abstract should be limited to 150 words, under the following headings:

Objective - reflecting the purpose of the study or the hypothesis that is being tested

Study Design - the setting for the study, the subjects (number and type), the treatment or intervention, and the type of statistical analysis

Result - include the outcome of the study and statistical significance, if appropriate

Conclusion - state the significance of the results

Introduction

The Introduction should assume that the reader is knowledgeable in the field and should therefore be as brief as possible but can include a short historical review where desirable.

Materials / subjects and Methods

This section should contain sufficient detail, so that all experimental procedures can be reproduced, and include references. Methods, however, that have been published in detail elsewhere should not be described in detail. Authors should provide the name of the manufacturer and their location for any specifically named medical equipment and instruments, and all drugs should be identified by their pharmaceutical names, and by their trade name if relevant.

Results and Discussion

The Results section should briefly present the experimental data in

text, tables or figures. Tables and figures should not be described extensively in the text, either. The discussion should focus on the interpretation and the significance of the findings with concise objective comments that describe their relation to other work in the area. It should not repeat information in the results. The final paragraph should highlight the main conclusion(s), and provide some indication of the direction future research should take.

Acknowledgements

These should be brief, and should include sources of support including sponsorship (e.g. university, charity, commercial organization) and sources of material (e.g. novel drugs) not available commercially.

Conflict of interest

Authors must declare whether or not there is any competing financial interests in relation to the work described. This information must be included at this stage and will be published as part of the paper. Conflict of interest should also be noted on the cover letter and as part of the submission process. See the Conflict of Interest documentation in the Editorial Policy section for detailed information.

References

Only papers directly related to the article should be cited. Exhaustive lists should be avoided. References should follow the Vancouver format. In the text they should appear as numbers starting at one and at the end of the paper they should be listed (double-spaced) in numerical order corresponding to the order of citation in the text. All authors should be quoted for papers with up to six authors; for papers with more than six authors, the first six only should be quoted, followed by *et al*. Abbreviations for titles of medical periodicals should conform to those used in the latest edition of *Index Medicus*. The first and last page numbers for each reference should be provided. Abstracts and letters must be identified as such. Papers in press and papers already submitted for publication may be included in the list of references but no citation is required for work that is not yet submitted for publication.

Journal article, up to six authors et al:

Martin JC, Bourgnoux P, Fignon A, Theret V, Antoine JM, Lamisse F et al. Dependence on human milk essential fatty acids on adipose stores during lactation. *Am J Clin Nutr* 1993; **58**: 653–569.

Journal article, e-pub ahead of print:

da Costa SP, van den Engel–Hoek L, Bos AF. Sucking and swallowing in infants and diagnostic tools. *J Perinatol* 2008; e-pub ahead of print 17 January 2008; doi:10.1038/sj.jp.7211924.

Journal article, in press:
Brown N. Perinatal and newborn care in South Asia: priorities for action. *Arch Dis Child*(in press).
Complete book:
Willett WC. *Nutritional Epidemiology*. Oxford University Press: New York, 1998.
Chapter in book:
Blizzard RM, Bulatovic A. (1996). Syndromes of psychosocial short stature. In: Lipshitz F (ed). *Pediatric Endocrinology*. Marcel Dekker: New York, 1986, pp 213–276.
Abstract:
Minck P. A synactive model of neonatal behavioral organization. *Phys Occup Ther Pediatr* 2002; **22**(Suppl 1): 28 (abstract 456).
Correspondence:
Sehgal A, Ramsden A (2008). Treating hypotension in the preterm infant: when and with what: a critical and systematic review [letter]. *J Perinatol* **28**, 167.
EndNote users should select the *The Journal of Perinatology* output style for the correct reference style.
Personal communications must be allocated a number and included in the list of references in the usual way or simply referred to in the text; the authors may choose which method to use. In either case authors must obtain permission from the individual concerned to quote his/her unpublished work.

Tables

These should be labelled sequentially and cited within the text. Each table should be presented on its own page, numbered and titled. Reference to table footnotes should be made by means of Arabic numerals. Tables should not duplicate the content of the text. They should consist of at least two columns; columns should always have headings. Authors should ensure that the data in the tables are consistent with those cited in the relevant places in the text, totals add up correctly, and percentages have been calculated correctly. Unlike figures or images, tables may be embedded into the word processing software if necessary, or supplied as separate electronic files.

Figures

Figures and images should be labelled sequentially, numbered and cited in the text. Figure legends should be brief, specific and appear on a separate manuscript page after the References section. Refer to (and cite) figures specifically in the text of the paper. Figures should not be embedded within the text. If a table or figure has been published before, the authors must obtain written permission to reproduce the material in both print and

electronic formats from the copyright owner and submit it with the manuscript. This follows for quotes, illustrations and other materials taken from previously published works not in the public domain. The original source should be cited in the figure caption or table footnote. The use of three-dimensional histograms is strongly discouraged when the addition of the third dimension gives no extra information. Scale markers should be used in the image for electron micrographs, and indicate the type of stain used. Detailed guidelines for submitting artwork can be found by downloading the Artwork Guidelines PDF.

Supplementary information

Supplementary information (SI) is peer-reviewed material directly relevant to the conclusion of an article that cannot be included in the printed version owing to space or format constraints. The article must be complete and self-explanatory without the SI, which is posted on the journal's website and linked to the article. SI may consist of data files, graphics, movies or extensive tables, view the Artwork Guidelines PDF for more information on accepted file types. Authors should submit documents in their FINAL format as they are not edited, typeset or changed, and will appear online exactly as submitted. When submitting SI authors are required to:

- Include a text summary (no more than 50 words) to describe the contents of each file.
- Identify the types of files (file formats) submitted.
- Include the text "Supplementary information is available at (the journal's name)'s website" at the end of the article and before the references.

House Style

General

- Do not make rules thinner than 1pt (0.36mm)
- Use a coarse hatching pattern rather than shading for tints in graphs
- Colour should be distinct when being used as an identifying tool
- Spaces, not commas should be used to separate thousands
- Abbreviations should be preceded by the words they stand for in the first instance of use
- Use SI units throughout
- Text should be double spaced with a wide margin
- At first mention of a manufacturer, the town (and state if USA) and country should be provided

Availability of data and materials

An inherent principle of publication is that others should be able to

replicate and build upon the authors' published claims. Therefore, a condition of publication is that authors are required to make materials, data and associated protocols available in a publicly accessible database (as detailed in the sections below on this page). Where one does not exist, the information must be made available to referees at submission and to readers promptly on request. Any restrictions on materials availability or other relevant information must be disclosed in the manuscript's methods section and should include details of how materials and information may be obtained.

Sequences, structures and 'omics': Papers reporting protein or DNA sequences and molecular structures will not be accepted without an accession number to Genbank/EMBL/DDBJ, Protein DataBank, SWISS-PROT or other appropriate, identified, publicly available database in general use in the field that gives free access to researchers from the date of publication.

Authors of papers describing structures of biological macromolecules must provide experimental data upon the request of editors if they are not already freely accessible in a publicly available database such as Protein DataBank, Nucleic Acids Database or Biological Magnetic Resonance Databank. Five separate copies of these data should be provided to the editors in an appropriate format (for example, CD or DVD) for the purposes of peer-review.

Abbreviations and Symbols

Do not use abbreviations in the title or abstract, and limit their use in the text. The first time an abbreviation appears it should be preceded by the words for which it stands. For a list of standard medical abbreviations and measurements, consult the *American Medical Association Manual of Style: A Guide for Authors and Editors,* 9th edition (Baltimore: Lippincott, Williams & Wilkins, 1998). There should be no hyphenation of phrases such as "very low birth weight", "birth weight" and "extremely low birth weight".

Fast Track Publication

For manuscripts dealing with urgent issues that necessitate expedient publication, the author may request a Fast Track Review. The author should state the reason for such a request in his or her cover letter. The editor will make a decision for fast track within a few days. If an adverse decision is made then the authors may request withdrawal of the manuscript or allow processing in the normal fashion.