Title:

EXTENSIVELY DRUG-RESISTANT *ACINETOBACTER BAUMANNII* COMPLEX (XDR-AB) COLONISATION AND CLINICAL OUTCOME IN NEONATES: A RETROSPECTIVE CASE-CONTROL STUDY

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

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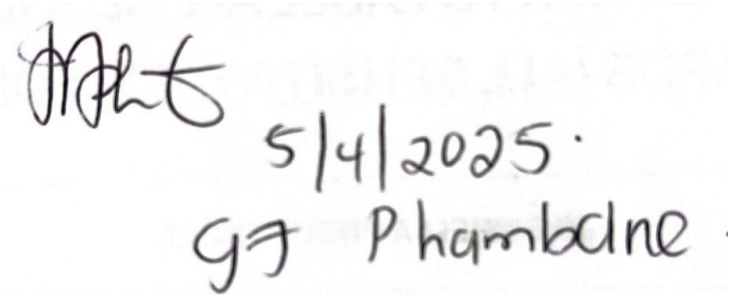
Candidate's declaration:

I Gugulethu Julia Phambane declare that this research report is my own, unaided work. It is being submitted for the Degree of Master of Medicine in Clinical Microbiology at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



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Dedication

I want to express my heartfelt gratitude to my family and friends for being pillars of support: Matshedisho Ellen Phambane, Sphesihle Donald Phambane, Lehlogonolo Ephraim Phambane, Dimpho Ephraim Phambane, Thabo Lawrence Phambane, Kgaugelo George Phambane, Peter Japtha Djiane, and Precious Fhumulani Munyai.

Presentations arising from this study

The research will be presented as a poster at the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Global Congress 2025 in Vienna, Austria, from 11–15 April 2025. In addition, an oral presentation of the findings will be delivered at the Infection Control Africa Network (ICAN) Congress 2025, scheduled to take place in Cape Town, South Africa, from 29 June to 2 July 2025.

Publications

This research work has not yet been published but will be submitted for publication in the future.

Abstract

Extensively drug-resistant *Acinetobacter baumannii* (XDR-AB) is a significant cause of healthcare-associated infections, particularly in neonatal intensive care units (NICUs). This study aimed to determine the prevalence of XDR-AB colonisation and the odds of subsequent invasive infection. The secondary objective was to identify risk factors and in-hospital outcomes. We investigated 485 neonates admitted at Rahima Moosa Mother and Child Hospital from 1 June 2020 to 31 December 2022. Among them, 116 (23.92%) were colonised with XDR-AB, with a median colonisation age of 4 days. Of these, 29 (25%) developed invasive disease, and 12 (10.34%) died. Mechanical ventilation increased the risk of colonisation by 2.28 times. The study highlights the high prevalence of early colonisation and associated mortality, emphasizing the need for screening, vigilant monitoring, and tailored antibiotic treatment for colonized neonates

Keywords: Extensively drug-resistant *Acinetobacter baumannii*, colonisation, invasive disease, risk factors

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Nomenclature/List of abbreviations and symbols

Abbreviations:

XDR: Extensively drug-resistant *Acinetobacter baumannii*

RMMCH: Rahima Moosa Mother and Child Hospital

NICU: Neonatal intensive care unit

Definitions

1. **XDR-AB** is an *A. baumannii* isolate non-susceptible to at least one agent in all but two or fewer antimicrobial categories

2. **XDR-AB colonisation** is having XDR-AB cultured from a screening swab (rectal or skin) after 72 hours of admission.

3. **XDR-AB non-colonised** is a participant who had a negative XDR-AB screening swab during admission.

4. **Neonatal sepsis:** Sepsis within 28 days of life, usually presents with non-specific clinical features or laboratory signs of sepsis with positive microbiological culture from a sterile site. Blood culture is the gold standard for diagnosis.

5. **Antibiotic exposure** means a patient has received at least one antibiotic before a positive screening swab or culture.

6. **Invasive XDR-AB disease**: first positive culture of XDR-AB from a sterile site (blood culture and/or cerebrospinal fluid sample).

CHAPTER 1: INTRODUCTION

1.1. Introduction

This chapter discusses the background of XDR-AB colonisation as a burden in healthcare-associated settings and the challenges in the management of XDR-AB invasive disease. It covers the problem statement, objectives, and a detailed literature review of the study.

1.2. Background

Healthcare-associated infections (HAIs) pose a substantial burden, resulting in prolonged hospital stays, limited treatment options, increased health costs, and heightened morbidity and mortality (5,6). Neonates possess immature immune systems, while critically ill patients may have impaired, suppressed, or dysregulated immune responses. Consequently, both groups are at a heightened risk for sepsis (4). Globally, neonatal sepsis ranks as the third most common cause of neonatal mortality, with antimicrobial resistance (AMR) accounting for approximately 31% to 63% of these deaths (5,7).

Healthcare-associated infection rates are twice as high in developing countries compared to developed countries, with a prevalence of 12.8% in Africa (6). The prevalence of HAIs in neonatal intensive care units (NICUs) in Africa was estimated at 62.5% in 2010 and 27% in 2022, with over 40% of HAIs attributable to *A. baumannii* (6).

The rise in AMR among HAIs results in an estimated 700,000 deaths annually, a figure projected to reach 10 million deaths by 2050 (7). Antimicrobial resistance to first line and empiric treatments in hospitalised patients creates a deficit in antimicrobial options due to a lack of new drug developments (8). Multi-drug-resistant organisms (MDROs) are challenging to treat in low-to-middle-income countries (LMICs) due to resource limitations (6,9,10). Thus, knowledge of antimicrobial resistance and the pathogenesis of microorganisms is critical to address these challenges (9).

The World Health Organization (WHO) has declared AMR as one of the top ten global public health threats. Priority pathogens associated with HAIs and increased

AMR rates include the ESKAPE pathogens (*Enterococcus* species, *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* species*, and Escherichia coli)* (8). *Acinetobacter baumannii*, in particular, frequently causes outbreaks in NICUs worldwide (5,9). In a South African surveillance study by Perovic *et al*. (2017-2019) 41% of XDR-AB cultures were from children under 1 year (11).

In this study, we investigate the *Acinetobacter baumannii complex* (ABC), which includes *A. baumannii*, *A. pittii*, *A. nosocomialis*, and *A. calcoceticus* (11). This complex is characterised as non-glucose-fermenting, oxidase-negative, pleomorphic aerobic, gram-negative coccobacilli commonly found in soil, water, on the skins of healthy individuals, and in hospital environments (10,11). ABC may colonise invasive devices such as catheter ports, urinary catheters, and mechanical ventilation equipment without necessarily causing disease (9,11).

Invasive diseases caused by ABC include bacteraemia, meningitis, endocarditis, pneumonia (particularly ventilator-associated pneumonia), urinary tract infections, wound infections, soft tissue infections, osteomyelitis, ear and eye infections, peritonitis, and catheter-related infections (9,11,12). The literature identifies ventilator-associated pneumonia (VAP) and bloodstream infections as the most common HAIs caused by ABC (6,13).

Acinetobacter baumannii complex is particularly successful in causing HAIs due to its ability to acquire antimicrobial resistance and it is difficult to eradicate due to environmental persistence (2,9). Extensively drug-resistant *Acinetobacter baumannii* (XDR-AB) is defined as an isolate non-susceptible to at least one agent in all but two or fewer antimicrobial categories: aminoglycosides, carbapenems (meropenem, imipenem, doripenem), fluoroquinolones, piperacillin-tazobactam, extended-spectrum cephalosporins, trimethoprim-sulfamethoxazole, ampicillin-sulbactam, polymyxins and/or tetracyclines (1).

South African surveillance (2017-2019) revealed that at least 75% of the *A. baumannii* were XDR-AB, with the highest burden in Gauteng province (11). There is increasing resistance to gentamicin (57.2%) and cefotaxime (44.9%) among HAIs, which are used as part of empiric treatments for neonatal sepsis (3–5). The emergence of resistance to carbapenems (88% of isolates were found to be resistant

to meropenem), colistin (4%), and other drugs used to treat ABC infections is concerning (11).

Mechanisms of resistance in XDR-AB include β-lactamases (Class A to D), porin losses (Omp family, CarO), altered target sites (e.g. penicillin-binding proteins, lipopolysaccharides, DNA gyrase), efflux pumps, aminoglycoside-modifying enzymes (AMEs), among other mechanisms (9).

In South Africa, treatment options for XDR-AB infections are limited. Current options include the combinations of polymyxin, amikacin, high dose carbapenem, and tigecycline, but resistance to these agents is increasing (10). Novel drugs such as ampicillin-sulbactam, sulbactam-durlobactam, and eravacycline show promise but remain unaffordable in LMICs (12). In the South African public sector, the last resort for treating XDR-AB is colistin combined with a carbapenem. However, colistin poses risks of nephrotoxicity, and the emergence of colistin resistance remains a significant challenge (10). The global prevalence of colistin heteroresistance is approximately 33%, and a South African surveillance study conducted between 2017-2019 found colistin resistance in 4% of XDR-AB isolates among infants (11,14).

1.3. Problem statement

The neonatal wards at Rahima Moosa Mother and Child Hospital have been affected by recurring outbreaks of XDR-AB infections. Consequently, screening for XDR-AB was implemented for neonates admitted to neonatal wards and NICU during an outbreak, and routine surveillance continued after the outbreak. Screening in the NICU was prioritised because of the increased prevalence of XDR-AB invasive disease before the study period. The antimicrobial resistance profile of XDR-AB complicates the selection for empiric treatments, posing significant challenges for clinicians. Hence, it is essential to investigate the relationship between XDR-AB colonisation and subsequent invasive disease, as well as the factors influencing colonisation in neonates.

1.4. Justification

Extensively drug-resistant *Acinetobacter baumannii* (XDR-AB) is a major cause of infectious disease outbreaks in NICU and poses a significant public health threat (3). There is a scarcity of studies on the risk factors for XDR-AB colonisation in neonates, particularly in South Africa. Understanding the impact of XDR-AB colonisation on in-hospital neonatal outcomes in the NICU can guide empiric treatment for suspected sepsis, necessitate active surveillance, and inform infection and prevention control strategies. Furthermore, early identification of XDR-AB colonisation could be the cornerstone for a cost-effective prevention strategy in neonatal care.

1.5. Research question

What proportion of neonates have XDR-AB colonisation, and how many develop subsequent invasive disease?

1.6. Aim of the study

To determine the prevalence of XDR-AB colonisation and progression to invasive infection in neonates admitted at RMMCH, Johannesburg.

1.7. Study objectives

Primary objectives

1. To determine the prevalence of XDR-AB colonisation in the neonates who were screened.
2. To determine the prevalence of invasive XDR-AB infections in neonates colonised and non-colonised with XDR-AB.

Secondary objectives

1. To determine in-hospital outcomes of neonates colonised with XDR-AB.
2. To determine the risk factors associated with XDR-AB colonisation in neonates.

1.8. Literature review

Extensively drug-resistant *Acinetobacter baumannii* (XDR-AB) colonisation and infection of neonates, particularly in neonatal intensive care units (NICU), is a concern, significantly impacting antimicrobial therapy. The colonisation rate among preterm neonates ranged from 12% to 21.4%, particularly in the first week of life (4,15–17). Notably, 57% of the neonates who were colonised remained so for the duration of their NICU stay, which was associated with prolonged hospitalisation (18).

Colonisation is postulated to precede infection and is a critical risk factor for subsequent invasive infection and poor prognosis (2). Bacterial translocation from the gut and immature defence mechanisms in premature neonates are linked to neonatal sepsis (4). Gut colonisation significantly increases the risk of blood-stream infections with XDR-AB (RR 3.6) (4). Approximately 7.9% to 25% of colonised neonates in NICU develop invasive disease, including bloodstream infections (4,17,19). In contrast, only 5.8% of the non-colonised neonates develop invasive infections (4). A South African study found about 41% of XDR-AB-associated bloodstream infections in infants (11).

Although *A. baumannii* has traditionally exhibited fewer virulence factors and lower attributable mortality compared to other gram-negative bacteria, recent findings suggest the emergence of hypervirulent strains with high antimicrobial resistance and increased mortality (9). Khurana *et al*. found a post-colonisation mortality risk of 26%, with cases of XDR-AB bloodstream infections preceded by colonisation showing a high mortality rate of 75% (4). However, the overall risk of mortality was higher in the colonised group at 6 months. Additionally, Perovic *et al*. reported that 36% of neonates infected with XDR-AB died during hospitalisation (11). Mortality was significantly higher in infected neonates compared with those merely colonised (39% vs. 9.5%, p= 0.01) (16). More studies noted a higher likelihood of invasive disease post-colonisation in patients requiring invasive ventilation, previous antibiotics, renal replacement therapy, cardiovascular disease, and extended ICU stays, most cases attributable to skin and mucosal barrier impairment (2,4,13). Mortality risk was particularly high in the infected neonates with lower birth weight (mean 960g to 1685g) and younger age (mean 6 days) (16).

The significant risk factors associated with XDR-AB colonisation at admission include younger age, male gender, small gestational age, and respiratory distress requiring

any form of ventilation (4,16,17). Additionally, mechanical ventilation and caesarean section were identified as significant risk factors for colonisation by drug-resistant organisms (15). In adults, the use of permanent devices (OR 10), mechanical ventilation (OR 40.01), a urinary catheter (OR 1.96), and carbapenem use (OR 5.39) are linked with colonisation (20). Suspected sepsis, prolonged ventilation, and prolonged ICU stay are also significant factors (2).

Literature indicates that preterm neonates are rapidly colonised during vaginal delivery, breastfeeding, from maternal periodontal flora, contaminated environmental surfaces, procedures, and human contact (4). Early identification of XDR-AB carriage allows for the timely implementation of infection control measures, preventing further spread and identifying patients at risk of subsequent XDR-AB infection (8).

In a country burdened by HIV/AIDS, there is a lack of studies assessing the association between HIV infection and *A. baumannii* acquisition or its link with mortality. This literature review has highlighted research gaps in neonates, including the prevalence of colonisation, subsequent invasive infection and risk factors associated with colonisation.

1.9. Conclusion

This chapter has covered the background, objectives and rationale of conducting this study.

CHAPTER 2: METHODS AND MATERIALS

2.1. Introduction

This chapter covers the methods and materials used in the study.

2.2. Ethics

The Human Research Ethics Committee (HREC), University of Witwatersrand, Johannesburg, approved this study (Approval number M23092; see Appendix 8.3). Permission was obtained from the Head of Clinical Microbiology and Infectious Disease Department (CMID) at the University of Witwatersrand, the Head of the Paediatrics Department Rahima Moosa Mother and Child Hospital (RMMCH), and the National Health Research Database (NHRD) of the Department of Health, and National Health Laboratory Services (NHLS).

The patient's identifiers and raw data were kept confidential, accessible only to the statistician and supervisors. Written consent was not required as this was a retrospective study, and the patients remained anonymous.

2.2. Study design

A retrospective case-control study was conducted in the neonatal wards (ward 16B and NICU) on neonates admitted from 1 June 2020 to 31 December 2022 at RMMCH, a specialised academic regional hospital in Johannesburg, South Africa, which serves West Rand and regions B and C of the Johannesburg Metropolitan Ward Council. The study period was chosen due to the availability of medical records. The hospital has approximately 15,000 deliveries and 2,300 neonatal admissions annually and has a bed capacity of 61 neonatal beds, including 10 neonatal intensive care units, 35 intermediate care, and 12 kangaroo mother care units.

Screening for XDR-AB colonisation was initiated in March 2019 during an outbreak in the unit, but routine surveillance continued even after the outbreak was declared over in July 2019. All neonates in the neonatal intensive care unit were screened on admission. In the other neonatal wards, only XDR *A. baumannii* exposed neonates were screened *i.e.*, if one neonate in the cubicle got XDR *A. baumannii infection*, then the rest of the neonates in the same cubicle would be screened and were

cohorted according to their screening swab results. Each neonate had one rectal swab and one skin screening swab (the skin swab was used to swab both the groin and the axillae).

1. Laboratory data of all XDR-AB screening swabs (rectal and/or skin) was retrospectively extracted from the NHLS Corporate Data Warehouse CDW database to calculate colonisation prevalence.

2. XDR-AB invasive infections were determined from the NHLS CDW database using the first positive culture from a sterile site (blood culture and/or CSF). The neonates who did not have cultures taken from sterile sites had no evidence of sepsis.

3. Clinical details and in-hospital outcomes were obtained from the RMMCH neonatal database.

4. The different datasets were cleaned and merged using STATA software, utilising patient identifier numbers to integrate the different datasets.

2.3. Study population and sample

We recruited 485 participants for this study. Figure 2.1 illustrates the sampling procedure for the study.

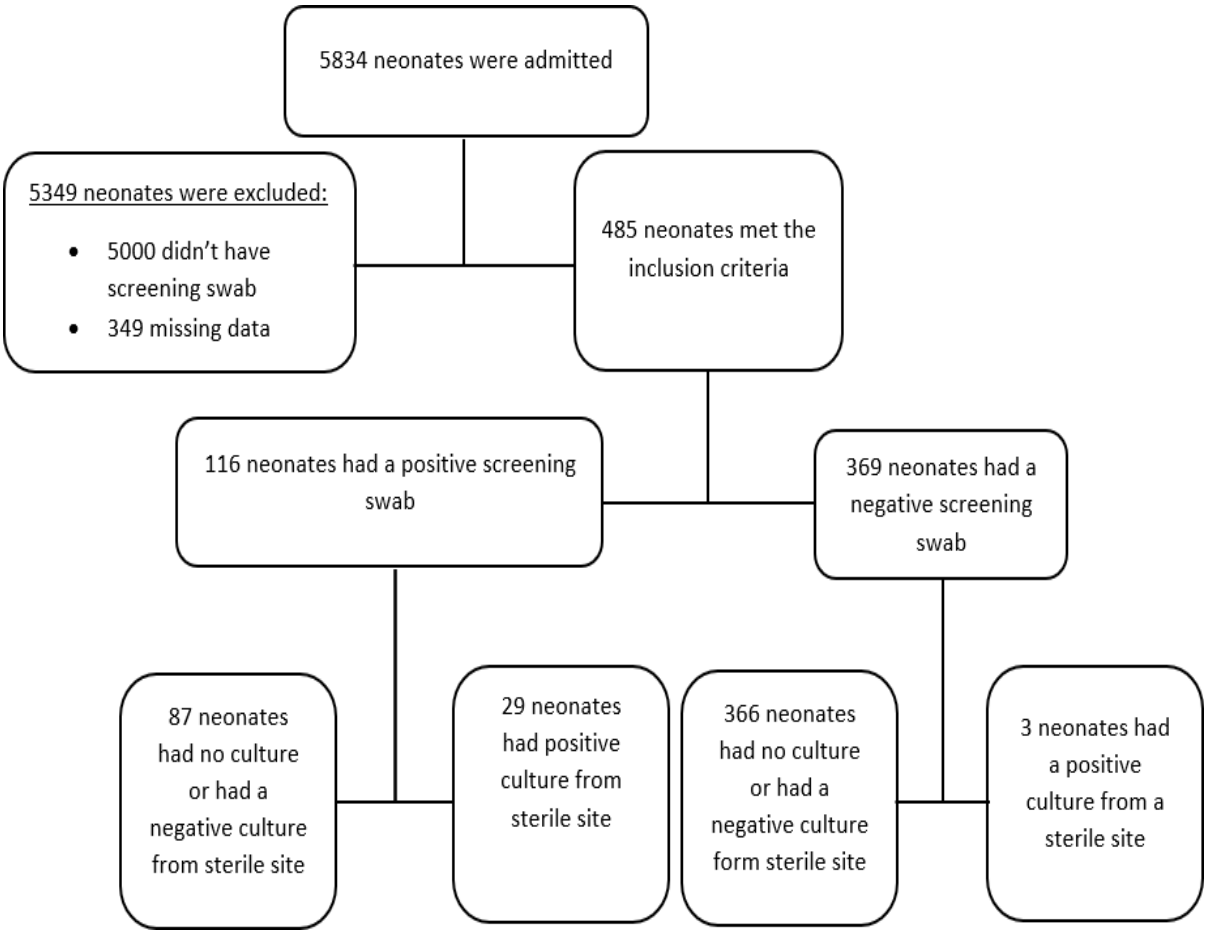

Figure 2.1: The flow of participants during the study

2.4. Study setting

Inclusion criteria: All admitted neonates (less than or equal to 28 days) admitted at RMMCH neonatal wards (16B and NICU) who had an XDR-AB screening swab (skin and rectal swab) between 1 June 2020 and 31 December 2022. Only the first positive screening swab was considered; subsequent positives were not counted. Patients with a second positive XDR-AB screening swab during readmission were not considered a separate case.

Exclusion criteria: Patients with missing medical records or clinical data.

Note: Clinical data for invasive devices other than mechanical ventilation, mixed feeds, and length of stay was missing and thus not analysed.

2.5. Data collection

Data source and selection: Data on XDR-AB colonisation and invasive disease was sourced from the NHLS CDW data and met the definition criteria of XDR-AB. Clinical information was gathered from the RMMCH database. Individual datasets were cleaned and merged into a single dataset with clearly defined variables.

Variables:

Independent variables: XDR-AB screening result, sex, birth weight, gestational age, age at testing, HIV exposure, HIV PCR results, surgical history, invasive devices, ICU admission, length of ICU stay, antibiotic exposure, diagnosis at admission, mode of delivery, and type of feed.

Dependent variables: in-hospital outcome (alive, discharged home, discharged to other wards, died, referred to another facility, or unknown), and XDR-AB invasive disease.

Outcome measures:

Primary objective: Number of neonates colonized with XDR-AB and the proportion developing the subsequent invasive disease in the colonised and non-colonised neonates. Measured variables: XDR-AB screening swab results and XDR-AB invasive disease.

Secondary objective: Measured variables for factors associated with colonisation: age, gestational age, sex, birth weight, HIV exposure, HIV PCR results, surgical history, ICU admission, antibiotic exposure, diagnosis at admission, mode of delivery, type of feeds and mechanical ventilation, and in-hospital outcomes

2.6. Statistical methods

Demographics and methodology: Baseline demographics and clinical characteristics of participants were assessed using descriptive statistics. Continuous variables were assessed for normality using kernel density distribution and presented as median and interquartile range (IQR). Age was analysed with the Mann-Whitney U

test. Categorical variables were presented as frequency (number, percentage) and compared using the Pearson Chi-square test.

Objective 1: Determine the prevalence of XDR-AB colonisation in neonates using frequency and percentages.

Objective 2: Assess the prevalence of invasive XDR-AB infections in colonised neonates using frequency and percentages and compared using the Pearson Chi-square test.

Objective 3: Evaluate in-hospital outcomes of neonates colonised with XDR-AB, presented as frequency and percentages, and compared using the Pearson Chi-square test.

Objective 4: Identify factors associated with XDR-AB colonisation in neonates.

1. Use logistic regression to determine unadjusted ratios for each factor variable.
2. Adjust for confounding factors using logistic regression to determine adjusted odds ratios for age, gestational age, sex, birth weight, HIV exposure, HIV PCR results, surgical history, ICU admission, antibiotic exposure, diagnosis at admission, mode of delivery, type of feeding, and mechanical ventilation.
3. Use stepwise logistic regression to determine the factors that are significantly associated with XDR-AB colonisation. A probability value of 0.15 was used for selection.

All models were assessed for the goodness of fit. Data analysis was performed using STATA version 18.0 software (StataCorp. 2023. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP). A p-value of <0.05 was considered statistically significant.

2.7. Conclusion

A detailed explanation of the study design, ethics and statistical methods to be used in the analysis has been provided.

CHAPTER 3: RESULTS

3.1. Introduction

This section discusses all the findings and interpretation of the study results.

3.2. Results

Demographics of the study population and prevalence of XDR-AB colonisation

This study included 485 participants. The baseline clinical characteristics and demographics of the participants screened for extensively drug-resistant *Acinetobacter baumannii* (XDR-AB) colonisation are illustrated in Table 1. Of the 485 participants, 116 (23.92%) were colonised with XDR-AB, while 369 (76.08%) were not. The gender distribution was similar across both groups. The median age of participants was 4 days, with an interquartile range of 2 to 7 days. Notably, 8.5% of neonates had very low birth weight (<1000g), and the percentage of neonates with a gestational age of less than 28 weeks was higher in the colonised group (9.5%) compared to the non-colonised group (3.5%). There were significant differences in the birth weight and gestational age between colonised and non-colonised groups (p-value=0.003 and p-value=0.009, respectively) (Table 3.1).

Table 3.1: Demographic description and prevalence of XDR-AB colonisation in neonates at RMMCH				
	XDR-AB colonisation			
	Non-colonised	**Colonised**	**Total**	**p-value**
Number of participants	369(76.1%)	116 (23.9%)	485 (100.0%)	
Age in days	4.0 (2.0- 7.0)	4.0 (2.9- 7.0)	4.0(2.0 -7.0)	0.941
Sex				0.682
Female	173 (46.9%)	54 (46.6%)	227 (46.8%)	
Male	134 (36.3%)	46 (39.7%)	180 (37.1%)	
Unknown	62 (16.8%)	16 (13.8%)	78 (16.1%)	
Gestational age				0.009
< 28 weeks	13 (3.5%)	11 (9.5%)	24 (4.9%)	
28-32 weeks	111 (30.1%)	40 (34.5%)	151 (31.1%)	
33-37 weeks	72 (19.5%)	27 (23.3%)	99 (20.4%)	
> 37 weeks	97 (26.3%)	16 (13.8%)	113 (23.3%)	
Unknown	76 (20.6%)	22 (19.0%)	98 (20.2%)	
Birth weight (grams)				0.003
<1000g	22 (6.0%)	19 (16.4%)	41 (8.5%)	
1000g- 1500g	81 (22.0%)	28 (24.1%)	109 (22.5%)	
1500g- 2000g	101 (27.4%)	28 (24.1%)	129 (26.6%)	
>2500g	165 (44.7%)	41 (35.3%)	206 (42.5%)	
Ventilation				<0.001
No	188 (50.9%)	39 (33.6%)	227 (46.8%)	
Yes	105 (28.5%)	55 (47.4%)	160 (33.0%)	
Unknown	76 (20.6%)	22 (19.0%)	98 (20.2%)	
Surgical history				0.192
No	293 (79.4%)	93 (80.2%)	386 (79.6%)	
Yes	0 (0.0%)	1 (0.9%)	1 (0.2%)	
Unknown	76 (20.6%)	22 (19.0%)	98 (20.2%)	
Type of feeding				0.765
Breast milk	190 (51.5%)	64 (55.2%)	254 (52.4%)	
Formula	12 (3.3%)	3 (2.6%)	15 (3.1%)	
Unknown	167 (45.3%)	49 (42.2%)	216 (44.5%)	
ICU admission				0.615
No	101 (27.4%)	29 (25.0%)	130 (26.8%)	
Yes	268 (72.6%)	87 (75.0%)	355 (73.2%)	
Antibiotic exposure				0.016
No	83 (22.5%)	13 (11.2%)	96 (19.8%)	

Yes	210 (56.9%)	81 (69.8%)	291 (60.0%)	
Unknown	76 (20.6%)	22 (19.0%)	98 (20.2%)	
Admission diagnosis				0.033
Sepsis	9 (2.4%)	4 (3.4%)	13 (2.7%)	
CNS pathology	33 (8.9%)	12 (10.3%)	45 (9.3%)	
GIT pathology	27 (7.3%)	15 (12.9%)	42 (8.7%)	
Lung pathology	71 (19.2%)	33 (28.4%)	104 (21.4%)	
Cardiovascular pathology	7 (1.9%)	4 (3.4%)	11 (2.3%)	
Genetic disorder	1 (0.3%)	1 (0.9%)	2 (0.4%)	
Liver pathology	2 (0.5%)	0 (0.0%)	2 (0.4%)	
Unknown	219 (59.3%)	47 (40.5%)	266 (54.8%)	
Mode of delivery				0.301
Caesarean section	145 (39.3%)	55 (47.4%)	200 (41.2%)	
Normal vaginal delivery	135 (36.6%)	37 (31.9%)	172 (35.5%)	
Unknown	89 (24.1%)	24 (20.7%)	113 (23.3%)	

There were 31 neonates (both colonised and non-colonised) from the entire study who had positive cultures collected from sterile sites subsequent to the surveillance cultures. The neonates who did not have cultures taken from sterile sites had no evidence of sepsis. Invasive disease was significantly more common among neonates colonised with XDR-AB. Twenty-nine (25%) colonised neonates developed invasive XDR-AB disease compared to 3 (0.81%) who were not colonised. The odds of invasive disease in the colonised neonates were 41 times higher (p value <0.001, 95% CI: 12.07-211.26) than the odds of invasive disease in non-colonised neonates, although the confidence interval was wide (Table 3.2).

Table 3.2: Prevalence of invasive XDR-AB infections in colonised and non-colonised neonates			
	Positive culture from sterile site (invasive disease)	**Negative culture or no sterile culture done**	**Total**
Colonised neonates (N, %)	29(25%)	87(75%)	116
Non-colonised neonates (N, %)	3(0.81%)	366(99.19%)	369
Total	**31**	**454**	**485**
	Point estimate		**95% CI**
Odds ratio	41		12.07-211.26

The outcomes for neonates colonised with XDR-AB showed significantly higher in-hospital mortality compared to non-colonised neonates (10.43% vs 1.36%, respectively, $p<0.001$). Most participants from both groups were discharged home (approximately 47%), with no major difference between the two groups. However, some non-colonised neonates were discharged to other wards (21.41% vs 14.43%) and a minority of the neonates were transferred to another facility (2.59% vs 2.17%). The status of 129 participants from both groups remains unknown. There is strong evidence indicating a significant association between XDR-AB colonisation and in-hospital mortality, with colonised neonates having 7.72 times higher odds of dying compared to the non-colonised group ($p<0.001$, 95% CI: 2.61-22.89). Other outcomes did not show a significant association with colonisation status (Table 3.3).

Table 3.3: The relationship between in-hospital outcomes in colonised and non-colonised neonates						
Outcome	**Colonised neonates (n=116)**	**Non-colonised neonates (n=369)**	**Total**	**Odds ratio**	**p-value**	**95% Confidence interval**
Died	12(10.34%)	5(1.36%)	17(3.51%)	7.72	0.000	2.61-22.89
Discharged home	55(47.41%)	177(47.97%)	232(47,84%)	0.69	0.234	0.38-1.27
Transfer to other wards	17(14.66%)	79(21.41%)	96(19.79%)	0.69	0.787	0.31-4.71
Transfer to another facility	3(2.59%)	8(2.17%)	11(2.27%)	1.211	0.792	0.56-1.56
Unknown	29(25%)	100(27.10%)	129(26.60%)	0.93	0.792	0.23-0.42

Key findings on logistic regression include antibiotic exposure which increased the risk of colonisation by 2.46 times (p=0.006; 95% CI: 1.30-4.66) and the presence of mechanical ventilation that increased the risk of colonisation by 1.94 times (p=0.028; 95% CI:1.08-3.49). However, antibiotic exposure was found to be influenced by confounding or effect modification (Table 3.4).

Table 3.4: Logistic regression model accounting for confounding in factor variables with XDR-AB colonisation

		XDR-AB COLONISATION					
		UN-ADJUSTED			ADJUSTED		
Factors	**Label**	**OR**	**p**	**95% CI ( )**	**OR**	**p**	**95% CI ( )**
Age in days		1.00	0.941	0.96-1.04	1.8	0.280	0.92-1.23
Sex	Female	ref					
	Male	1.10	0.681	0.70-1.73	1.18	0.554	0.69-2.01
ICU admission	No	Ref					
	Yes	1.13	0.62	0.70-1.82	1.04	0.87	0.61-1.79
Admission diagnosis	Sepsis	baseline					
	CNS pathology	0.82	0.667	0.31-6.18	1.39	0.667	0.31- 6.18
	GIT pathology	1.25	0.954	0.24-4.44	1.04	0.954	0.24-4.44
	Lung pathology	1.05	0.831	0.30- 4.55	1.16	0.831	0.30-4.55
	CVS pathology	1.29	0.824	0.19-7.82	1.23	0.824	0.19-7.82
	Genetic disorder	2.25	0.418	0.21-132.53	5.28	0.311	0.21-132.53
	Liver pathology	1			1		
Antibiotic exposure	No	baseline					
	Yes	2.46	0.006	1.30-4.66	1.22	0.50	0.56-2.64
HIV exposure	No	Ref					
	Yes	1.56	0.12	0.89-2.70	0.76	0.52	0.33-1.74
HIV PCR results	Negative	baseline					
	Positive	1			1		
	Indeterminate	1			1		
Birth weight	<1000g	Ref					
	1000g-1500g	0.40	0.017	0.19-0.85	0.61	0.311	0.24-1.58
	1500g-2000g	0.32	0.003	0.15- 0.67	0.47	0.152	0.16-1.33
	>2000g	0.29	0.001	0.14-0.58	0.74	0.653	0.20-2.71
Mechanical ventilation	No	baseline					
	Yes	2.53	0.000	1.57-4.06	1.94	0.028	1.08-3.49
Gestational age	<28 weeks	baseline					
	28-32 weeks	0.43	0.057	0.18-1.03	0.41	0.124	0.13- 1.28
	33-37 weeks	0.44	0.082	0.18-1.10	0.60	0.435	0.16- 2.18
	>37 weeks	0.20	0.001	0.07-0.51	0.20	0.040	0.05-0.93
Surgical history	No	baseline					
	Yes	1			1		
Type of feeding	Breastmilk	baseline					
	Formula	0.74	0.652	0.20-2.71	0.47	0.311	0.11-2.01
Mode of delivery	Caesarean section	baseline					
	Normal vaginal delivery	0.72	0.183	0.45-1.17	0.68	0.171	0.39- 1.18

A stepwise logistic regression model identified significant associations with XDR-AB colonisation, achieving a likelihood ratio of 58.30 and p-value of 0.0002 with a good model fit (p-value= 0.3326). There was an association between XDR-AB colonisation and gestational age of more than 37 weeks (p-value=0.003) as well as the presence of mechanical ventilation (p-value= 0.001). Our logistic regression model found that a gestational age of more than 37 weeks was protective against XDR-AB colonisation (OR=0.23, p-value=0.003, 95% CI= 0.086-0.61), and the presence of mechanical ventilation increased the risk by 2.28 times (p=0.001 and 95% CI:1.38-3.76) (Table 3.5).

Table 3.5: A final logistic regression model showing factors that have a significant association with XDR-AB colonisation

Factors	Label	Odds ratio	p-value	95% CI
Gestational age	<28 weeks	Baseline		
	28-32 weeks	0.36	0.030	0.14-0.91
	33-37 weeks	0.41	0.064	0.16-1.05
	>37 weeks	0.23	0.003	0.086-0.61
Mechanical ventilation	No			
	Yes	2.28	0.001	1.38-3.76

Factors such as age, sex, admission diagnosis, antibiotic exposure, birth weight, type of feeding, ICU admission, and surgical history did not show a significant association with XDR-AB colonisation in the final analysis (Supplementary Table 6.1).

3.3. Conclusion

This chapter has covered the findings in detail and tables have been included with all the data analysed obtained.

CHAPTER 4: DISCUSSION AND CONCLUSION

4.1. Introduction

This chapter covers the discussion of the results obtained in comparison to the literature available. It also covers the limitations, strengths of the study, conclusion as well as the recommendations based on the findings.

4.2. Discussion

This study aimed to determine the prevalence of XDR-AB colonisation and its progression to invasive disease in neonates at RMMCH from 1 June 2020 to 31 December 2022. The guts of neonates are colonised with XDR-AB from their mothers and the environment shortly after birth (4). The median age of neonates in this study was 4 days, indicating early colonisation. RMMCH neonatal wards showed a high burden of XDR-AB colonisation at 23.92%, compared to 12 to 21.4% in the literature, highlighting the significant issue of healthcare-associated infections (HAIs) in neonatal wards (4,15–17).

After colonisation, the bacterium can translocate into the bloodstream, leading to invasive disease due to immature defence mechanisms in neonates (4). Our study showed a significant association between colonisation and increased risk of invasive disease among neonates (25%, OR 41, p-value <0.001). Meschiari *et al*. demonstrated that subsequent XDR-AB invasive disease risk increases more than eightfold with colonisation (20). Other studies reported that approximately 7.9% to 25% of colonised neonates in NICU develop invasive disease, including bloodstream infections (4,17,19).

Our study found a neonatal mortality rate of 10.34% in the colonised group, which is lower than the 26% mortality risk reported in the literature (RR 1.9, 95% CI 0.8-4.4) (4). Although the outcome of 26% of neonates was unknown, low mortality in our setting could be due to the care by highly specialised neonatologists and daily ward rounds involvement of infectious disease specialists and microbiologists as well. Ferreira *et al.* explored the outcomes of neonates colonised with multi-drug-resistant organisms and found a mortality rate of 12.8%, similar to our study findings (21). Although our study did not examine mortality outcomes amongst neonates with XDR-AB invasive disease, previous research has shown that the mortality in neonates is

significantly higher in XDR-AB bloodstream infections reaching 75%, but the sample was small (4). The available data in the South African setting focuses mostly on outcomes in infected neonates and not on the outcomes of colonised neonates.

Our logistic regression model found that gestational age of more than 37 weeks was protective against XDR-AB colonisation. This aligns with Arhoune *et al.* who identified younger gestational age as a risk factor for XDR-AB colonisation (17)**.** Mechanical ventilation emerged as a significant risk factor for XDR-AB colonisation in neonates (OR 2.06, p-value=0.009, 95% CI 1.20-3.56). Zheng *et al.* also identified mechanical ventilation for more than 5 days as a significant risk factor for XDR-AB colonisation, affecting 83.1% of the participants (2). The literature suggests that medical instruments can harm the protective mucosa of the respiratory tract during mechanical ventilation, leading to alterations in the air pressure and haemodynamic that increase the risk of multidrug-resistant *A. baumannii* infection (22). Meschiari *et al.* further demonstrated that mechanical ventilation increased XDR-AB colonisation by 40 times in adults, highlighting its importance in both adults and neonates (20).

Antibiotic exposure was identified as a significant risk factor for colonisation in our study before adjusting for confounding factors. Previous research by Meschiari *et al.* demonstrated that antibiotic exposure, especially to carbapenems, was a significant risk factor for colonisation with XDR-AB (OR 5.39) (20). In contrast, Arhourne *et al.* evaluated the exposure to combination therapy of ampicillin-gentamicin or ceftriaxone-gentamicin as a risk factor for intestinal colonisation with XDR-AB and found that these exposures were not statistically significant (17). This conflicting evidence highlights the need for further research on antibiotic exposure as a predictor of colonisation in neonates.

Unknown mode of delivery, admission diagnosis, HIV PCR, and HIV exposure were identified as significant factors associated with colonisation in the logistic regression model. However, due to insufficient data, definitive conclusions could not be drawn from these factors. Mode of delivery was found to be a marginally significant risk factor of intestinal colonisation before adjusting for confounding variables, but in the final analysis it did not reach statistical significance (17). Delivery by caesarean section has been identified as a significant risk factor for colonisation in the first week of life in neonates (p=0.04) (15). Zheng *et al.* found that an admission diagnosis of suspected sepsis, hepatobiliary and pancreatic disease were significant factors associated with colonisation, illustrating the need to explore admission diagnosis (2).

HIV-positive status was not a significant risk factor for mortality in a surveillance study conducted in South Africa; only 14% of the persons with XDR-AB bacteraemia were HIV-positive, noting that 41% of the population were infants (11). In a study conducted in adult ICU, HIV infection was not found to be a significant factor associated with *A. baumannii* colonisation or infection (23). Limited literature is available investigating HIV exposure and HIV infection as risk factors for XDR-AB colonisation in neonates. Future studies at the same facility and other centres should explore these variables further.

Limitations of the study

This single-centre retrospective study, conducted in a setting with a high burden of XDR-AB may not be generalisable to other settings. The study design did not allow for an exploration of risk factors related to infection prevention control strategies. As a retrospective study, it excluded urine and respiratory samples as it was difficult to distinguish whether positive XDR-AB results indicated colonisation or invasive disease. The wide confidence interval for XDR-AB colonisation leading to XDR-AB invasive disease, suggests that a larger sample size would have provided more certainty. The study encountered limitations due to substantial amounts of missing data, e.g. gender, gestational age and a substantial number of participant outcomes were unknown. In the final model, unknown mode of delivery, HIV PCR, HIV exposure, and admission were significant factors of colonisation, hence, they could not be evaluated further. These gaps in data could potentially impact the validity and accuracy of the study results. Additionally, the absence of clinical data for the use of invasive devices (other than mechanical ventilators), mixed feeds, and length of stay limited the assessment of the impact of these variables on XDR-AB colonisation. Long-term mortality after colonisation could not be assessed due to the lack of follow-up data after discharge.

Strengths of the study

This study focused solely on the risk factors of XDR-AB colonisation, eliminating potential confounding from invasive disease. This approach allowed for a detailed investigation of factors associated with XDR-AB colonisation and its associated risk factors in neonates within the South African region.

4.3. Conclusion

The study demonstrated that most neonates were colonised with XDR-AB at an early age in this unit. A high prevalence of XDR-AB colonisation was observed in the RMMCH neonatal wards. XDR-AB colonisation significantly increased the risk of developing invasive disease amongst neonates. In-hospital mortality was notably higher in the colonised group as compared to non-colonised neonates. The presence of mechanical ventilation was found to be associated with an increased risk of colonisation with XDR-AB.

This study supports that early identification of XDR-AB colonisation could predict neonates at risk of progression to XDR-AB invasive disease and subsequent mortality. We recommend XDR-AB screening neonates in this unit for neonates who are mechanically ventilated. High vigilance for invasive disease should be maintained when sepsis occurs in colonised neonates. Empiric antibiotic treatment should be tailored to cover the XDR-AB in neonates known to be colonised with XDR-AB since invasive disease is associated with high mortality.

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APPENDICES

Supplementary table

Table A: Other factors that were not significantly associated with XDR-AB colonisation				
	XDR-AB screen			
	Non-colonised	Colonised	Total	p-value test
Number of participants	369 (76.1%)	116 (23.9%)	485 (100.0%)	
Mode of delivery				
Caesarean section	145 (39.3%)	55 (47.4%)	200 (41.2%)	0.301
Normal vaginal delivery	135 (36.6%)	37 (31.9%)	172 (35.5%)	
Unknown	89 (24.1%)	24 (20.7%)	113 (23.3%)	
ICU admission				
No	101 (27.4%)	29 (25.0%)	130 (26.8%)	0.615
Yes	268 (72.6%)	87 (75.0%)	355 (73.2%)	
HIV exposure				
No	228 (61.8%)	64 (55.2%)	292 (60.2%)	0.289
Yes	55 (14.9%)	24 (20.7%)	79 (16.3%)	
Unknown	86 (23.3%)	28 (24.1%)	114 (23.5%)	
Type of feeding				
Breast milk	190 (51.5%)	64 (55.2%)	254 (52.4%)	0.765
Formula	12 (3.3%)	3 (2.6%)	15 (3.1%)	
Unknown	167 (45.3%)	49 (42.2%)	216 (44.5%)	
Surgical history				
No	293 (79.4%)	93 (80.2%)	386 (79.6%)	0.192
Yes	0 (0.0%)	1 (0.9%)	1 (0.2%)	
Unknown	76 (20.6%)	22 (19.0%)	98 (20.2%)	

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a retrospective case-control study

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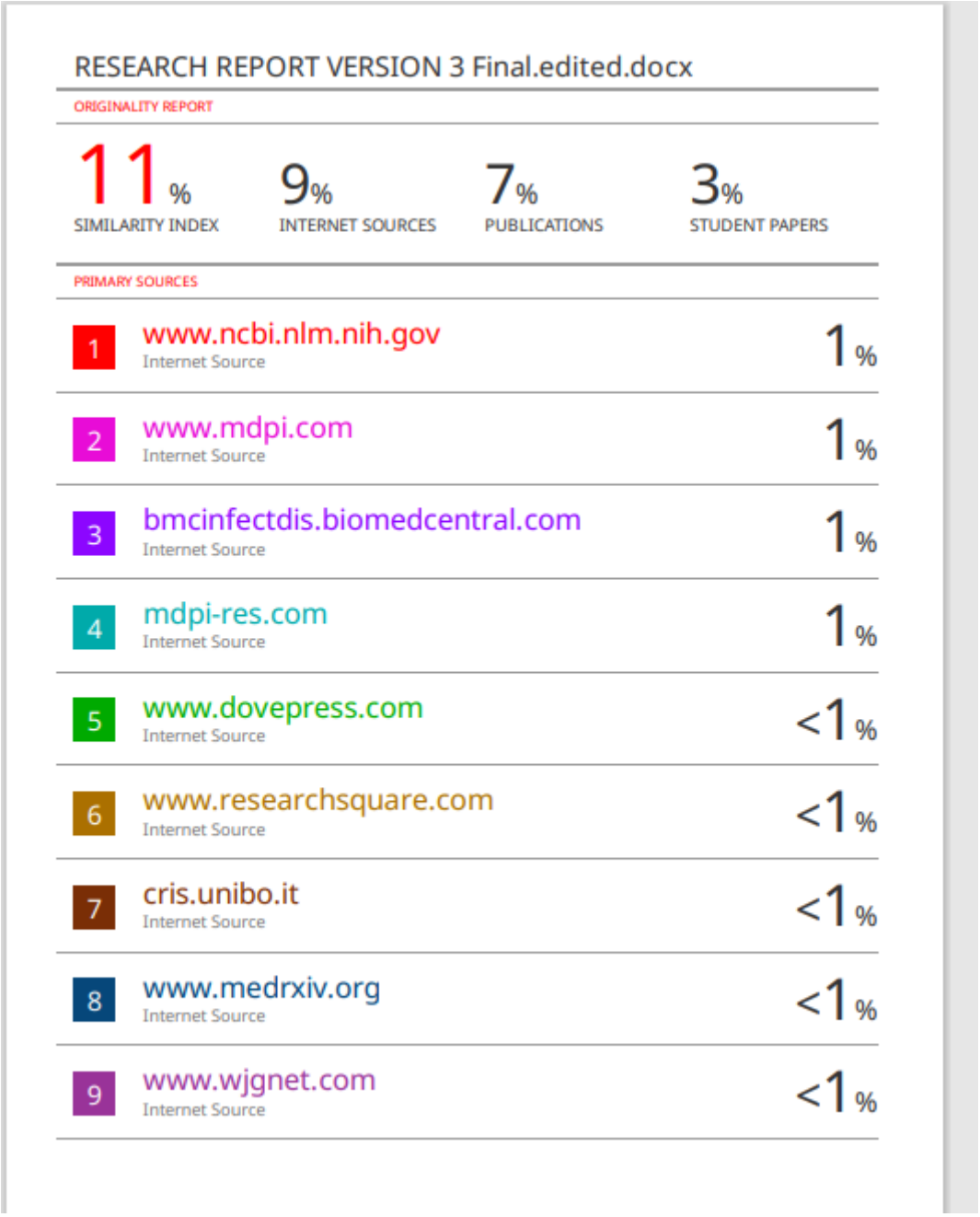
Student number: 985740

Degree: Master of Medicine in Clinical Microbiology and Infectious Disease

Institution: Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa



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Student: Gugulethu Julia Phambane

Student number: 385740

Degree: Master of Medicine in Clinical Microbiology

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Date: 2024/11/06

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Office of the Deputy Vice-Chancellor (Research and Innovation)

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FROM:	Mr Iain Burns Human Research Ethics Committee (Medical) Tel: 011 717 1252 E-mail: Iain.Burns@wits.ac.za
DATE:	10 January 2024
REF:	R14/49
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NAME: Dr GJ Phambane **DEGREE:** M Med
(Principal and Co-Investigators)

DEPARTMENT: School of Pathology
Dept of Clinical Microbiology & Infectious Diseases
Medical School
University

PROJECT TITLE: *Extensively drug-resistant Acinetobacter baumannii (XDR-AB) colonisation and clinical outcome in neonates: a retrospective case-control study*

DATE CONSIDERED: Ad hoc

DECISION: Approved unconditionally

CONDITIONS:

NOTE: If contact information regarding student study participants is required, please contact the Registrar's office <Nicoleen.Potgieter@wits.ac.za>

SUPERVISOR: Drs R Chomba, J Fredericks and S Ndzabandzaba

APPROVED BY:
Ms M van Niekerk, Co-Chairperson, HREC (Medical)

DATE OF APPROVAL: 10 January 2024 **EXPIRY DATE:** 9 January 2029

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12.1.2024
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PROTOCOL

Extensively Drug-Resistant *Acinetobacter baumannii* (XDR-AB) colonisation and clinical outcome in neonates: a retrospective case-control study

Researcher: Gugulethu Julia Phambane,

Student number: 385740,

Degree: MMED in Clinical Microbiology and Infectious disease,

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1. Definitions

1. Extensively Drug-Resistant *Acinetobacter baumannii* (XDR-AB) is defined as an isolate non-susceptible to at least one agent in all but 2 or fewer than 3 antimicrobial categories (1).

2. XDR-AB colonisation is defined as having XDR-AB cultured from a screening swab (rectal or nasopharyngeal or skin) during hospital admission (2).

3. XDR-AB non-colonized neonate is a neonate who had a negative XDR-AB screening swab during admission.

4. Neonatal sepsis: Sepsis within 28 days of life usually presents with non-specific clinical features of sepsis or laboratory signs of sepsis with positive microbiological culture from a sterile site. Blood culture is the gold standard for diagnosis (3).

5. Antibiotic exposure means a patient has received at least one antibiotic before a positive screening swab or culture

2. Background

Healthcare-associated infections (HAIs) are a major global burden and are associated with prolonged hospital stays, limited treatment options, increased health costs, and increased morbidity and mortality (4–6). Amongst hospitalised patients, neonates and critically ill patients have a large burden of sepsis due to an imbalanced immune system (3). Neonatal sepsis is rated the third global cause of neonatal mortality, of which antimicrobial resistance (AMR) accounts for about 31% to 63% of deaths (3). Africa has a high prevalence of HAIs in neonatal intensive care units (NICUs), estimated at 62.5% in 2010 and 27% in 2022 (4).

There is a rise in antimicrobial resistance amongst HAIs (1,4). Antimicrobial resistance has been estimated to cause 700 000 deaths annually (4). It is predicted to increase to 10 million deaths in 2050 (4). Antimicrobial resistance to the first line and empiric antimicrobial treatment in hospitalised patients creates a deficit in antimicrobial choice (1–3). There is a challenge of rising antimicrobial resistance, but

few new drug developments are in the pipeline (2,4,6). Multi-drug-resistant organisms (MDROs) are difficult to treat in low to middle-income countries (LMICs) due to a lack of resources and associated outbreaks in hospitals (6,7). Knowledge of antimicrobial resistance and the pathogenesis of pathogens remains critical to address these challenges (8).

The World Health Organization (WHO) has declared AMR as one of the top ten global public health threats (5). WHO has a list of priority pathogens in a healthcare setting; ESKAPE (*Enterococcus species*, *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter species, and Escherichia coli)* are the common HAIs with increased rates of antimicrobial resistance (5,7). *A. baumannii* is a common isolate that causes outbreaks in NICUs worldwide (7). A South African surveillance study by Perovic et al conducted between 2017 and 2019 found that 41% of the study population who cultured XDR-AB were children less than 1 year (5). It is difficult to eradicate drug-resistant *A. baumannii* strains due to their extensive drug resistance and persistence in the environment
(8).

Acinetobacter baumannii is the most isolated HAI in the *Acinetobacter baumannii* complex compared to *A. pittii*, *A. nosocomialis*, and *A. calcoceticus* (5). *A. baumannii* is a nonglucose-fermenting, oxidase-negative, pleomorphic aerobic, gram-negative coccobacilli (8).

Acinetobacter baumannii is an environmental pathogen found in soil, water, skins of healthy people, and the hospital environment (5). *Acinetobacter baumannii* colonises invasive devices such as catheter ports, urinary catheters, and mechanical ventilation equipment without causing disease (6,9). Invasive disease from *A. baumannii* includes bacteremia, meningitis, endocarditis, pneumonia (mechanical ventilator-associated pneumonia), urinary tract infections, wound infections, soft tissue infections, osteomyelitis, ear and eye infections, peritonitis, and catheter-related infections (5,6). Ventilator-associated pneumonia (VAP) is the most common HAIs caused by *A. baumannii* (9).

Colonisation is postulated to precede an infection and is considered an important risk factor for subsequent invasive infection (10,11). Gut colonisation with XDR *A. baumannii* is associated with an increased risk of blood-stream infection (10).

Bacterial translocation from the gut and immature defence mechanisms in premature neonates are linked to the development of neonatal sepsis from gut colonisation (10). XDR *A. baumannii* colonisation rate is 12 to 18% amongst preterm neonates in the first week of life and about 21% to 80% of the neonates who are colonised with XDR A. *baumannii* developed the invasive disease (9–11).

The risk factors of *A. baumannii* colonisation include previous admission to a long-term health care facility, recent invasive surgery, presence of co-morbidities, low socioeconomic status (overcrowding and lack of resources such as water), and previous use of carbapenems, fluoroquinolones and third generation cephalosporin (5,12).

A study by Khurana *et al* found that patients who required invasive (mechanical) ventilation and longer ICU (Intensive Care Unit) stay were more likely to be colonised with XDR-AB infections and had a substantial risk of death (11). Patients can be colonised with *A. baumannii* from the early days of their ICU stay and the risk increases with prolonged hospital stay (2). A study by Reddy *et al* found that all of the participants who had *A. baumannii* infection had received mechanical ventilation (9). This is attributable to the fact that skin and mucosal barrier impairment lead to pathogen colonisation (2). Reddy *et al* found that it was difficult to determine if prolonged hospital stay and the need for ventilation were due to A*. baumannii* acquisition or if they predisposed them to acquire the infection (9). A study done in Serbia illustrated that preterm neonates are rapidly colonised with XDR *A. baumannii* during delivery by caesarean section compared to vaginal delivery (13).

The presence of medical devices, antibiotic therapy, low birth weight, and preterm delivery were associated with invasive infections and high mortality rates (10).

A study by Magiorakos et al found that HIV-positive status is not a significant risk factor for mortality but the limitation in this study was that HIV status for about 31% of the participants was unknown (1). In a country where HIV is a major burden, there are limited studies that assess the association between HIV infection and A. *baumannii* acquisition and its association with mortality (14).

A. baumannii has been previously described to have few virulence factors compared to other gram-negative bacteria and with low *A. baumannii* attributable mortality (8).

Hypervirulent strains have been described that are associated with high antimicrobial resistance and mortality in individuals with minor underlying diseases (15).

Cases of XDR *A. baumannii* bloodstream infection preceded by colonisation have been found to have a high mortality rate of 75% (10). Mortality in neonates was found to be significantly higher in XDR-AB-infected patients than in those who were only colonised (39% vs. 9.5%, p= 0.01) (16).

There is increasing resistance to ampicillin, gentamicin, and cefotaxime among HAIs, which are used as empiric treatment for neonatal infections (7). Multi-drug resistant *Acinetobacter baumannii* (MDR-AB) is defined as an isolate non-susceptible to at least 1 agent in 3 or more antimicrobial categories (1). The last resort treatment for MDR-AB is carbapenems (5). The concern is emerging antimicrobial resistance against carbapenems, colistin, and other drugs in *A. baumannii* (17). The first carbapenem-resistant AB (CR-AB) was documented in 1991 and its prevalence has been increasing over the years (18). Three types of carbapenem resistance genes have been reported to drive carbapenem resistance in *A. baumannii* including bla_{NMD} (New Delhi metallobeta-lactamases), $bla_{\text{OXA-48}}$ and bla_{ADC} (5,18). In Africa, *blaOXA-48* is the common determinant of CR-AB infections amongst neonates and infants (5,18).

There are limited antibiotic options for CR-AB in the public health sector which include carbapenems, minocycline, colistin, and tigecycline (8). Treatment options for CR-AB involve the combination of polymixin, amikacin, high-dose carbapenem, and tigecycline with increasing resistance to these agents (8). Novel drugs such as ceftazidime-avibactam, ceftolozane-tazobactam, and eravacycline are promising treatment options but remain unaffordable in low- and middle-income counties (LMICs) (19).

In the South African public hospital setting, the last resort for treatment of XDR *A. baumannii* is colistin in combination therapy with a carbapenem (1). Colistin belongs to the polymyxin group (polymyxin A, B, C, D, and E) (17). Colistin possesses a risk of nephrotoxicity and the emergence of colistin resistance is a challenge (18). The global prevalence of colistin heteroresistance is 33% (20).

A South African surveillance study conducted between 2017 and 2019 found colistin resistance to be 4% in XDR-AB isolates among infants (5). Prior colistin treatment is associated with the selection of resistant subpopulations (20). The emerging colistin

resistance leaves clinicians with limited/no treatment options, especially in low resource countries (5),

XDR-AB is the leading cause of neonatal ICU outbreaks, and it is a public health threat (3). There are limited studies on the risk factors of colonisation with XDR-AB especially in neonates in South Africa. Available studies have not been done locally and have limitations.

Understanding the impact of XDR-AB colonisation on the prognosis of neonates in the NICU can assist in treatment, surveillance, and infection control strategies.

Aim of the study:

To determine the frequency of XDR-AB colonisation, subsequent invasive infections, and clinical outcomes in neonates colonised with XDR-AB at RMMCH (Rahima Moosa Mother and Child Hospital).

Study objectives

Primary objectives

1. To determine the XDR-AB colonisation frequency in the neonates
2. To determine the frequency of invasive XDR-AB infections in neonates colonised with XDR-AB.
3. To determine the in-hospital outcome of neonates colonised with XDR *A. baumannii* at the end of hospital stay (alive at discharge/demised/referral to another facility).

Secondary objectives

1. To determine the risk factors associated with invasive XDR-AB infections in neonates.

2. Methods

2.1. Study design

XDR-AB screening is done during outbreaks to determine who is colonised so as to institute infection prevention and control measures. The identification of XDR-AB from a screening swab, blood culture, and cerebrospinal fluid culture in the laboratory was done by planting the sample directly onto MacConkey agar and incubating agar plates aerobically for 24 hours (22). Non-lactose fermenting colonies which are typically circular, convex, smooth, pink colonies were isolated (22). Automated methods or biochemical tests were used for identity and antibiotic susceptibility testing of the isolate. XDR-AB was identified if the isolate was non-susceptible to at least one agent in all but 2 or fewer antimicrobial categories (9).

A case-control study will be conducted to retrospectively review the RMMCH neonates' medical records from wards 16B, High Care, and NICU. The laboratory data of all XDR-AB screening swabs will be extracted retrospectively from the NHLS (National Health Laboratory Service) database to calculate the frequency of XDR-AB colonisation.

The study period will be from 1 June 2020 to 31 December 2022.

The first positive culture of XDR-AB from a sterile site (blood culture or cerebrospinal fluid sample) will be used to diagnose invasive disease. The data of positive XDR-AB from sterile sites will be extracted from the NHLS database to determine the frequency of invasive disease in colonised and non-colonised neonates. The expected frequency of invasive disease is approximately 21%.

The medical records will be reviewed to determine clinical outcomes in the XDR-AB colonised participants compared to the control group. The neonate's medical records will be followed up until discharge from the hospital. The outcome will be assessed as either alive at discharge, demised, referred to another facility, or unknown if the notes are missing.

The medical records will be reviewed to record the different clinical variables (See addendum 1 and study setting).

2.2 Study population and sample:

All neonates who had a positive XDR-AB screening swab in the neonatal wards at RMMCH will be included in the study. The medical records and screening swabs must be done between 1 June 2020 and 31 December 2022. The expected positive screening swabs (case group) are 160 during the sampling period. The control group number (negative screening swab) will be matched to the neonates with a positive screening swab (expected controls 160); it must be a neonate with the same date of birth (+/- 7 days) and had a screening swab for XDR-AB taken on the same date (+/- 7 days) as the case patient.

2.3. Study setting

Inclusion criteria: All admitted neonates with an XDR-AB screening swab (skin swab and/or nasopharyngeal swab and/or rectal swab) from neonatal wards (16B, High care, and NICU) between 1 June 2020 to 31 December 2022. The reason for the choice of this period is the availability of stored records.

Exclusion criteria: Patients over 28 days of life at the time of the screening swab, patients who lack medical records, and patients without an XDR-AB screening swab.

Only the first positive screening swab will be considered so subsequent positive screening swabs will not be counted. Patients who had a second positive rectal swab during readmission to the neonatal wards would not be considered a separate study case.

2.4. Data collection

The data will be extracted from the NHLS database system and patients' records at Rahima Moosa Mother and Child Hospital using the data collection tool (Addendum 1). The following variables will be extracted: All XDR-AB screening swabs, all blood cultures, and cerebrospinal fluid cultures results of all neonates from appropriate wards during the study period. The patient identifiers will be required to match screening swab results with the culture results (See addendum 1 for the data collection sheet).

Variables:

Independent variables: XDR-AB screening swab result, age, gender, birth weight, gestational age, chronological age, HIV exposure, HIV PCR results, previous surgical history, invasive devices, admission to ICU, length of ICU stay, antibiotic exposure, diagnosis at admission, mode of delivery and type of feeds.

Dependent variables: in-hospital outcome (discharged alive, discharged to another hospital facility, unknown or demised), XDR-AB invasive disease

Confounders: low-birth weight, prematurity, co-morbidities

2.5. Data analysis

Variables will be summarised as count (percentages, mean (standard deviation), and median (25th–75th percentiles) as appropriate. Shapiro-Wilk test and Q-Q probability plot for verification will be implemented to assess the normal distribution and ascertain skewness and kurtosis. For any departures from normal distributions, the data will be transformed before analysis, or tests that are less sensitive to departure from normal distribution will be used. Chisquare tests for categorical variables, independent T-tests for continuous variables, and Oneway Analysis of Variance (ANOVA) will be used to assess differences between more than two groups and further post-hoc Tukey tests will be implemented. The interrelation between continuous variables will be assessed via univariable and multivariable regression

models and categorical outcomes and models will be fitted to obtain odds ratios (OR) and 95% confidence interval (CI) and stratified by groups deemed necessary. Covariates in multilevel models will include age, gender, birthweight, gestational weight, HIV exposure, previous surgical history, invasive devices, length of stay in the ward, antibiotic exposure, and type of feeds. A p-value of 0.05 will be considered significant and 95 % confidence intervals (CI) stated. Refer to addendum 2.

2.6. Ethics

The Ethics approval of this study will be obtained from the Wits Medical Human Research

Ethics Committee (HREC). Approval from the Head of Clinical Microbiology and Infectious Disease Department (CMID) at the University of Witwatersrand will be obtained. Permission to conduct the study will be obtained from the research committee and the Head of the Paediactrics Department Rahima Moosa Mother and Child Hospital and the National Health Research Database (NHRD). Approval from National Health Laboratory Services will be obtained.

The patient's details will be kept anonymous by assigning participants a unique number; this will ensure confidentiality is maintained throughout the study. A separate Excel document with patient identifiers matched with a unique number (record number) will be used. The data will then be exported to an Excel sheet. To keep the participant's data safe, the Excel or/and data sheet will be locked with a password which will be shared with supervisors only.

3. Timing

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
Literature review														
Preparing protocol														
Protocol assessment														

Wits PGC meeting														
Ethics submission														
Ethics HRC meeting														
Ethics feedback														
Data collection														
Data analysis														
Writing up														

4. Funding

The researcher will bear the costs of the research project as there is little cost anticipated.

5. Problems anticipated and limitations.

Missing records**,** different hospital numbers/MRNs are usually assigned to patients making it difficult to retrieve their results from NHLS LIS, and antibiotic exposure outside the hospital will not be captured. The patient outcome cannot be followed up after being discharged home or discharge to their referral centre and there are no records for the admissions to the paediatric unit for any readmission of patients beyond the neonatal period.

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Addendum 1: Data collection sheet

Patient/record number										
<u>Demographics:</u> **Gestational age in weeks** □ <28 weeks • 28-32 weeks • 32-38 weeks • >38 weeks **Chronological age in days:** **Birth weight in grams** □ <28g • 1000g-1500g • 1500g-2500g • >2500g **Gender:** • Male/female/Undetermined										

Admission to ICU: Yes/No If yes, specify the number of days in the ICU:									
<u>Co-morbidities:</u> HIV exposure (Yes/No/unknown) HIV PCR results: Positive/Negative/Unknown/Indeterminate									
<u>Mode of delivery:</u> Vaginal/Caesarian section									

Diagnosis at admission: Suspected sepsis Gastroenteritis Prematurity-related condition Other (Cardiovascular disease, Chronic renal disease, Lung pathology, metabolic); specify										
Type of feeds: • Breastmilk • Formula • Mixed feeding										

XDR-AB screening swab: Positive /Negative Day of life when swab taken/positive										
Sterile site culture Yes/No Day of Life on positive culture										
Antibiotic exposure Yes/No Specify: (Ampicillin/gentamicin, Cefotaxime Meropenem/imipenem Colistin Pen G Other)										
Invasive devices: **Yes/No** specify Ventilation (Yes/No) Intravascular devices (Yes/No)										

Surgical history **Yes/No** Date of operation Type of operation										
Clinical outcome at discharge: Discharged to ward Discharged home Referred to another hospital facility. Demised unknown										

Table 1. Frequency (%) of XDR-AB colonisation

	The overall number of swabs collected in the study period	XDR_AB colonised (n)	XDR-AB non colonised (n)
XDR_AB colonisation rate n (%)			

Table 2: Frequency (%) of invasive disease

	XDR-AB non-colonised patients n (%)	XDR-invasive disease from colonised patients n (%)	Odd ratio
XDR-AB invasive disease (n) %			
XDR-AB non-invasive disease **(n) %**			

Table 3: In-hospital outcome

	XDR-AB non- colonised (n %)	XDR-AB colonised (n%)

Clinical outcomes Discharged to ward Discharged home Referred to another hospital/facility Demised Unknown		

Table 4.1: Risk factors associated with XDR-AB invasive disease.

	XDR-AB colonised n (%)	XDR-AB colonised n (%)	p-value
Gender • Male • Female • Undetermined			
Gestational age in weeks • <28 weeks • 28-32 weeks • 32-38 weeks • >38 weeks			
Birth weight in grams <1000g • 1000g-1500g • 1500g-2500g • >2500g			

Procedures **<u>Invasive devices: Yes/No</u>** specify Ventilation (Yes/No) Intravascular devices (Yes/No) **<u>Surgical history</u>** **<u>Yes/No</u>** Date of operation Type of operation			
<u>Type of feeds:</u> • Breastmilk • Formula • Mixed feeding			

HIV exposure Yes NO Unknown			
HIV PCR Results Yes No Unknown Indeterminate			
Antibiotic exposure Yes /No Specify			

Diagnosis at admission: Suspected sepsis Gastroenteritis Prematurity-related condition			
Other (Cardiovascular disease, Chronic renal disease, Lung pathology, metabolic); specify			
Admission to ICU: Yes/No (n%)			

Table 4.2: Risk factors associated with XDR invasive disease.

	XDR-AB non colonised Mean, +-standard deviation/ median (Interquartile range)	XDR-AB deviation/ median colonised Mean, +-standard (Interquartile range)	p-value
Chronological age:			
Number of days in ICU:			