Developing an Antimicrobial Strategy for Sepsis in Malawi

-

Thesis submitted in accordance with the requirements of the Liverpool School of Tropical Medicine for the degree of Doctor in Philosophy by Joseph Michael Lewis

August 2019

Contents

List of Tables

List of Figures

Preface

Placeholder

Chapter 1

Introduction

Placeholder

1.1 Chapter Overview

1.2 Sepsis in sub-Saharan Africa

1.2.1 Search strategy

1.2.2 Defining sepsis

1.2.3 Applicability of sepsis-3 definitions in sub-Saharan Africa

1.2.4 Sepsis epidemiology in sub-Sahara Africa

1.2.4.1 Incidence

1.2.4.2 Risk factors: the sepsis population in sub-Saharan Africa

1.2.4.3 Outcomes

1.2.5 Sepsis aetiology in sub-Saharan Africa

1.2.5.1 Bacterial zoonoses, Rickettsioses and arboviruses

1.2.5.2 HIV opportunistic infections: PCP, histoplasmosis and cryptococcal disease

1.2.6 Sepsis management

1.2.6.1 Early goal directed therapy

1.2.6.2 Evidence to guide antimicrobial therapy in sSA

1.2.6.3 Evidence to guide intravenous fluid therapy in sub-Saharan Africa

1.3 ESBL-E in sub-Saharan Africa

1.3.1 Search strategy

1.3.2 Introduction: definition and classification of ESBL-E

1.3.3 Global molecular epidemiology of ESBL-E: an overview

1.3.3.1 1980s-1990s: First identificatiom of ESBL in nosocomial pathogens

1.3.3.2 1990s-2010s: Emergence and globalisation of CTX-M

Chapter 2

Methods

Placeholder

2.1 Chapter Overview

2.2 Study site

2.2.1 Malawi

2.2.2 Queen Elizabeth Central Hospital

2.2.3 Participating Laboratories

2.2.3.1 Malawi-Liverpool-Wellcome Clinical Research Programme

2.2.3.2 Malawi College of Medicine Tuberculosis Laboratory

2.2.3.3 Wellcome Trust Sanger Institute

2.3 Clinical Study

2.3.1 Entry Criteria

2.3.2 Study Visits and Patient Sampling

2.3.2.1 Enrollment assessment and first six hours

2.3.2.2 Subsequent visits

2.3.2.3 Blood, urine, and stool, sputum and CSF collection

2.3.2.4 Imaging: chest x-ray and ultrasound scanning

2.3.3 Outcomes and sample size calculations

2.4 Diagnostic Laboratory Procedures

2.4.1 Point of care diagnostics

2.4.2 Laboratory diagnostics

2.4.2.1 Haematology and biochemistry

2.4.2.2 Aerobic blood and CSF culture

2.4.2.3 Mycobacterial blood culture

Chapter 3

Mycobacterium tuberculosis BSI: an IPD meta analysis

Chapter 4

A clinical and microbiological description of sepsis in Blantyre, Malawi

Placeholder

4.1 Chapter overview

4.2 Introduction and chapter aims

4.3 Aims and Methods

4.4 Results

4.4.1 Study population

4.4.2 Symptoms and health-seeking behaviour

4.4.3 Admission physiology and laboratory investigations

4.4.4 Aetiology

4.4.5 Treatment

4.4.6 Outcome

4.4.7 Determinants of mortality

4.5 Discussion

4.5.1 Demographics and outcome: significant longer-term mortality

4.5.2 Aetiology: TB dominates as a cause of sepsis

4.5.3 Determinants of 28-day mortality: an expanded role for TB therapy?

4.5.4 Limitations

4.6 Conclusions and further work

4.7 Appendix

Chapter 5

Longitudinal ESBL-E carriage in Malawian adults in health and disease

5.1 Chapter Overview

5.2 Introduction and chapter aims

5.3 Methods

5.4 Results

5.4.1 Study population

In total, 425 participants were recuited to the study between 19th February 2017 and 2nd October 2018; 225 participants with sepsis (arm 1), 100 inpatients without antimicrobial exposure at baseline (arm 2) and 100 community members (arm 3). Flow of participants through the study is shown in Figure 5.2. It was often challenging to collect stool samples from participants but 87% (1416/1631) eligible patient-visits resulted in the collection of a stool sample. Drop out from the study and failure to collect stool samples were similar in arm 1 and 2 and with no apparent systematic bias, but both drop out and missing samples were less frequent in arm 3 (Figure 5.1A). There was siginifcant variation in the timing of stool sample collecion, with a distribution around the ostensible collation day (Figure 5.1B).

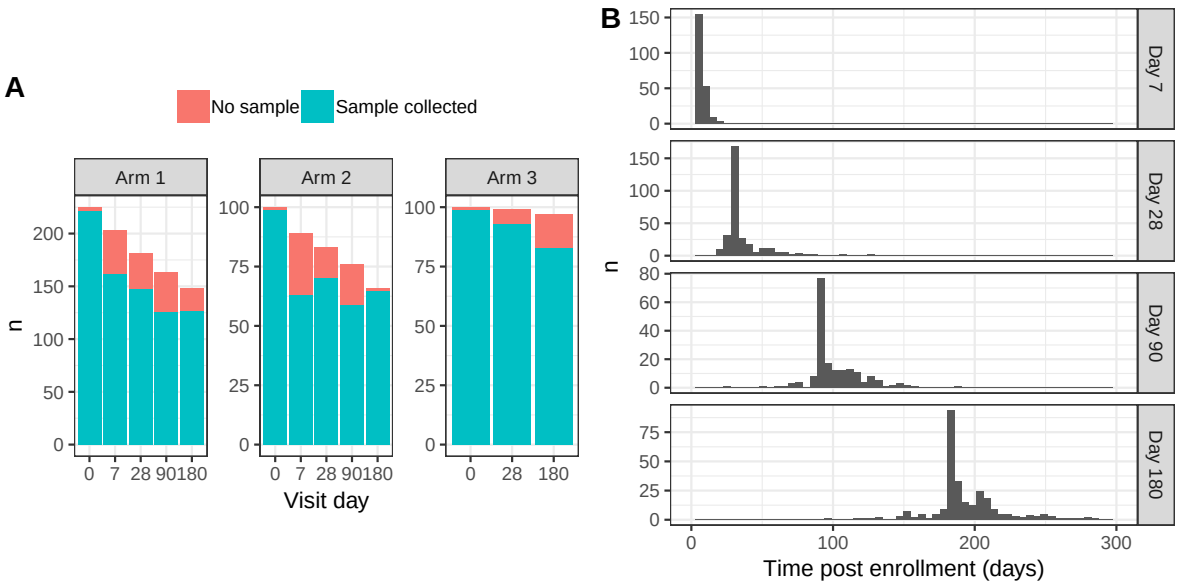

Figure 5.1: A: Missing stool samples stratified by arm and visit. Bar height at a given visit represents the number of eligible participants, coloured by successful sample collection (blue) or failure to collect a sample (red). B: Distribution of actual day of sample collection for ostensible day 7, 28, 90 and 180 samples showing considerable variation.

The baseline characetristics of the enrolled participants are shown in Table 5.1. There were some important differences between the arms of the study: despite matching on age and sex, antimicrobial-unexposed participants were older. They were also less likely to be HIV-infected than participants with sepsis (13% [12/89] of those with known HIV status were HIV-infected versus 67% [143/213] with sepsis), and less likely to have been treated for TB. Sepsis participants were more likely to have recieved antimicroials or been hospitalised in the previous 4 weeks. In the community arm of the study, there were a high proportion of participants (60% [60/100]) with an unknown HIV status, and there were some differences in toilet facilities, water sources, cooking fuel and presence of animals at home across the three groups.

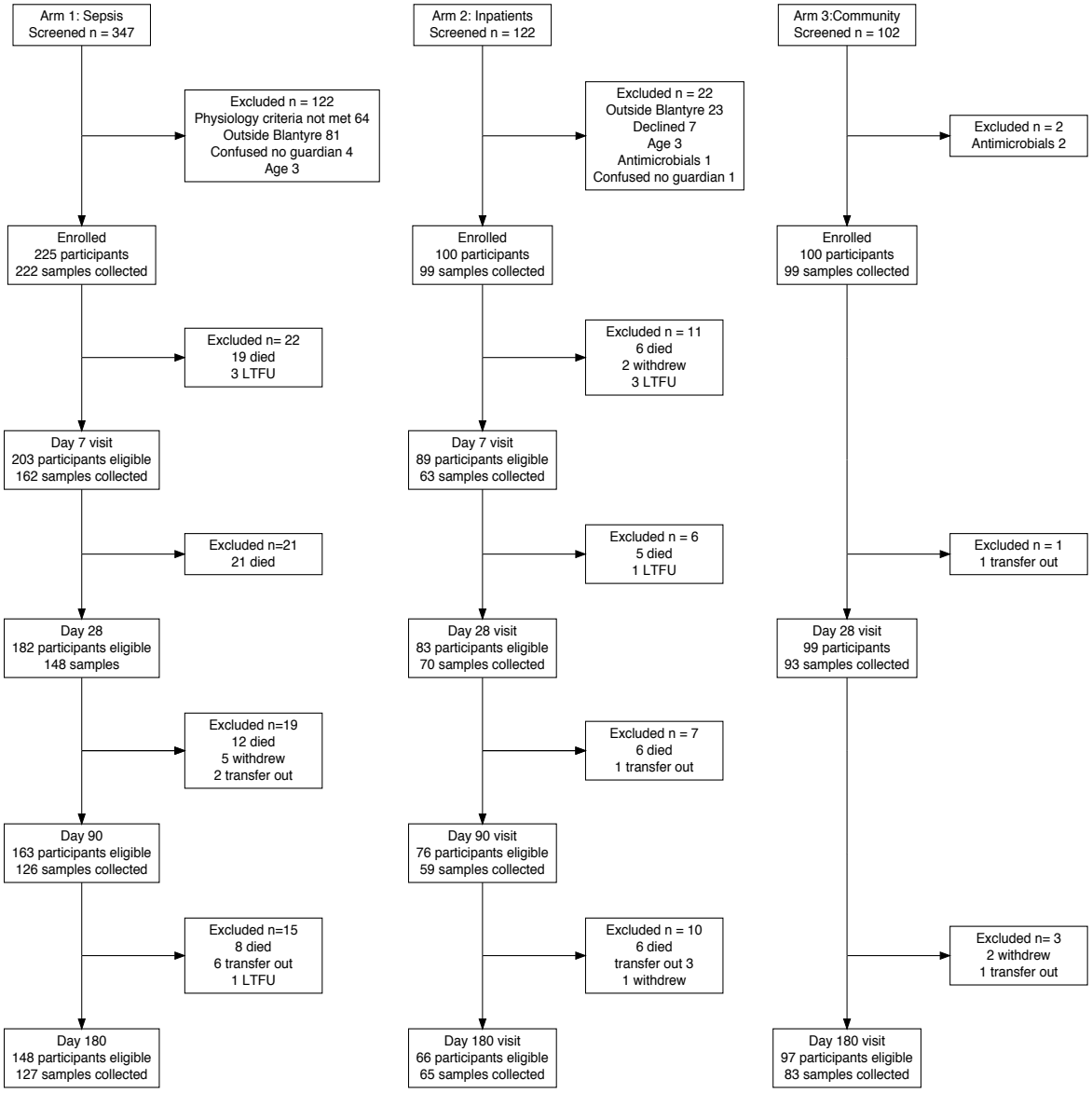

Figure 5.2: Study recruitment and follow up. At each time point *eligible participants* refers to participants who are known to be alive and have not withdrawn from the study by that time point, and *samples collected* refers to patients from whom a stool sample was sucessfully collected for that visit.

Table 5.1: Participant Characteristics

Variable	Sepsis	Inpatient	Community	p	Total
Demographics					
Age (yr)	**35.9 (27.8-43.5)**	**40.4 (29.1-48.3)**	**32.5 (24.0-38.4)**	**<0.001**	**35.6 (26.9-43.9)**
Male sex	114/225 (51%)	51/100 (51%)	40/100 (40%)	0.533	205/425 (48%)
HIV/TB status					
HIV Reactive	**143/225 (64%)**	**12/100 (12%)**	**18/100 (18%)**	**<0.001**	**173/425 (41%)**
HIV Non Reactive	**70/225 (31%)**	**77/100 (77%)**	**22/100 (22%)**	**<0.001**	**169/425 (40%)**
HIV Unknown	**12/225 (5%)**	**11/100 (11%)**	**60/100 (60%)**	**<0.001**	**83/425 (20%)**
Ever treated for TB	**37/225 (16%)**	**5/100 (5%)**	**4/100 (4%)**	**0.002**	**46/425 (11%)**
Of those, current TB treatment	10/37 (27%)	0/5 (0%)	4/4 (100%)	0.098	14/46 (30%)
ART status*					
Current ART*	117/143 (82%)	9/12 (75%)	18/18 (100%)	0.859	144/173 (83%)
Months on ART	28.7 (3.7-72.6)	35.1 (2.9-79.8)	31.5 (13.0-79.9)	0.693	29.5 (3.8-72.8)
ART regimen: EFV/3TC/TDF	110/117 (94%)	8/9 (89%)	17/18 (94%)	1.000	135/144 (94%)
ART status					
Current CPT†	98/141 (70%)	5/12 (42%)	7/18 (39%)	0.328	110/171 (64%)
Healthcare exposure last 4wk					
Antibiotics	**60/225 (27%)**	**0/100 (0%)**	**0/100 (0%)**	**<0.001**	**60/425 (14%)**
Hospitalised	**18/225 (8%)**	**1/100 (1%)**	**0/100 (0%)**	**0.001**	**19/425 (4%)**
Tobacco/alcohol use					
Never tobacco	196/225 (87%)	93/100 (93%)	90/100 (90%)	0.929	379/425 (89%)
Ex tobacco	17/225 (8%)	6/100 (6%)	2/100 (2%)	0.180	25/425 (6%)
Current tobacco	12/225 (5%)	1/100 (1%)	8/100 (8%)	0.070	21/425 (5%)
Current alcohol	51/225 (23%)	16/100 (16%)	18/100 (18%)	0.502	85/425 (20%)
Education					
Primary incomplete or complete	97/225 (43%)	50/100 (50%)	42/100 (42%)	0.739	189/425 (44%)
Some secondary education	47/225 (21%)	18/100 (18%)	30/100 (30%)	0.238	95/425 (22%)
Secondary school complete	48/225 (21%)	16/100 (16%)	19/100 (19%)	0.677	83/425 (20%)
No formal schooling	16/225 (7%)	13/100 (13%)	4/100 (4%)	0.094	33/425 (8%)
College or higher	17/225 (8%)	3/100 (3%)	5/100 (5%)	0.346	25/425 (6%)
Employment					
Unemployed	82/225 (36%)	34/100 (34%)	32/100 (32%)	0.866	148/425 (35%)
Self-employed	56/225 (25%)	32/100 (32%)	35/100 (35%)	0.325	123/425 (29%)
Currently employed	65/225 (29%)	26/100 (26%)	18/100 (18%)	0.269	109/425 (26%)
Student	21/225 (9%)	6/100 (6%)	15/100 (15%)	0.153	42/425 (10%)
Retired	1/225 (0%)	2/100 (2%)	0/100 (0%)	0.280	3/425 (1%)

Toilet facilities					
Pit latrine with slab +/- foot rest	**104/225 (46%)**	**25/100 (25%)**	**35/100 (35%)**	**0.039**	**164/425 (39%)**
Pit latrine with slab and cover +/- foot rest	**45/225 (20%)**	**19/100 (19%)**	**55/100 (55%)**	**<0.001**	**119/425 (28%)**
Hanging toilet/latrine	**59/225 (26%)**	**48/100 (48%)**	**9/100 (9%)**	**<0.001**	**116/425 (27%)**
Flush Toliet (any type)	14/225 (6%)	5/100 (5%)	1/100 (1%)	0.118	20/425 (5%)
No toilet	2/225 (1%)	2/100 (2%)	0/100 (0%)	0.533	4/425 (1%)
Composting toilet	1/225 (0%)	1/100 (1%)	0/100 (0%)	0.720	2/425 (0%)
Main water source					
Public tap/standpipe	**51/225 (23%)**	**8/100 (8%)**	**66/100 (66%)**	**<0.001**	**125/425 (29%)**
Piped outside dwelling	**69/225 (31%)**	**37/100 (37%)**	**9/100 (9%)**	**<0.001**	**115/425 (27%)**
Tube well/borehole	**64/225 (28%)**	**35/100 (35%)**	**15/100 (15%)**	**0.032**	**114/425 (27%)**
Piped into dwelling	30/225 (13%)	11/100 (11%)	7/100 (7%)	0.353	48/425 (11%)
Unprotected well/spring	5/225 (2%)	6/100 (6%)	2/100 (2%)	0.181	13/425 (3%)
Surface water (including rainwater collection)	4/225 (2%)	2/100 (2%)	0/100 (0%)	0.556	6/425 (1%)
Tube well with powered pump	2/225 (1%)	1/100 (1%)	1/100 (1%)	1.000	4/425 (1%)
Treat water with chlorine	**19/225 (8%)**	**5/100 (5%)**	**0/100 (0%)**	**0.004**	**24/425 (6%)**
No. household members					
Children	2.0 (1.0-3.0)	2.0 (1.0-3.0)	2.0 (1.0-3.0)	0.395	2.0 (1.0-3.0)
Adults	2.0 (2.0-3.0)	3.0 (2.0-4.0)	2.0 (2.0-4.0)	0.907	3.0 (2.0-4.0)
Electricty					
Electricity available in house	119/225 (53%)	41/100 (41%)	58/100 (58%)	0.357	218/425 (51%)
Main cooking fuel					
Charcoal	161/225 (72%)	63/100 (63%)	88/100 (88%)	0.291	312/425 (73%)
Wood	**61/225 (27%)**	**35/100 (35%)**	**11/100 (11%)**	**0.004**	**107/425 (25%)**
Electricity	3/225 (1%)	2/100 (2%)	1/100 (1%)	0.869	6/425 (1%)
Animals at home?					
Any animal	**71/225 (32%)**	**43/100 (43%)**	**15/100 (15%)**	**0.004**	**129/425 (30%)**
Poultry	46/71 (65%)	34/43 (79%)	10/15 (67%)	0.800	90/129 (70%)
Dogs	18/71 (25%)	11/43 (26%)	9/15 (60%)	0.201	38/129 (29%)
Other	11/71 (15%)	9/43 (21%)	5/15 (33%)	0.413	25/129 (19%)
Goats	12/71 (17%)	7/43 (16%)	1/15 (7%)	0.830	20/129 (16%)
Cattle	2/71 (3%)	3/43 (7%)	0/15 (0%)	0.406	5/129 (4%)

Note:

ART = Antiretroviral therapy, CPT = Co-trimoxazole preventative therapy, EFV: Efavirenz, 3TC: Lamivudine, TDF: Tenofovir. Numeric values are median (IQR)) unless otherwise stated. P-values are to assess for differened across the three groups: Fisher's exact test across the groups for categorical variable, and Kruskal-Wallace test for continuous variables.

* ART status includes HIV reactive only as denominator

† Missing CPT data for two participants.

5.4.2 Exposures druring the study period

Exposures to antimicrobials and hospitalisation of the cohort are shown in Figure 5.3 and Table 5.2. Antimicrobial-unexposed inpatients (Arm 2 participants) had a shorter length of hospital stay than participants with sepsis (Arm 1 participants): median (IQR) 2 (2-7) versus 5 (2-10) days, p = 0.002 by Kruskal-Wallace test. Five of the 100 Arm 2 participants were taking co-trimoxazole preventative therapy (CPT) at baseline, 18 received further courses of antimicrobials during the study period, and two were started on TB therapy. Some participants received combinations of these therapies, so in total 23% (23/100) Arm 2 participants received an antibacterial during the study period, mostly within 30 days following enrollment (Figure 5.3)), and most commonly ceftriaxone (Table 5.2).

Both antimicrobial exposure and hospitalisation were unusal in the community cohort; 7% (7/100) community (Arm 3) participants were taking CPT and one received a 5-day course of amoxicillin meaning that 8% (8/100) Arm 3 participants received an antibacterial during the study period. In addition one Arm 3 participant was hospitalised for 1 day in the study period. No Arm 3 participant received any TB therapy, and no Arm 2 or 3 participants received any antimalarial or antifungal therapy during the study period.

The most commonly receievd antibacterial by Arm 1 participants - those with sepsis - (apart from co-trimoxazole and TB therapy) was ceftrixaxone by some distance with 998 participant-days of expsoure in 189 participants during the study period, and a median 5 (IQR 3-7) day course. Ciprofloxacin and amoxicillin were also commonly received, with 61 participants receiving 398 participant-days of exposure to ciprofloxacin with a median 7 (IQR 5-7) day course, and 39 participants receiving 235 participant-days of exposure to amoxicillin with a median 5 (IQR 5-7) day course. Because of the chronic nature of the therapy, the greatest exposure (in terms of participant-days) were to co-trimoxazole and TB therapy, by an order of magnitude (Table 5.2).

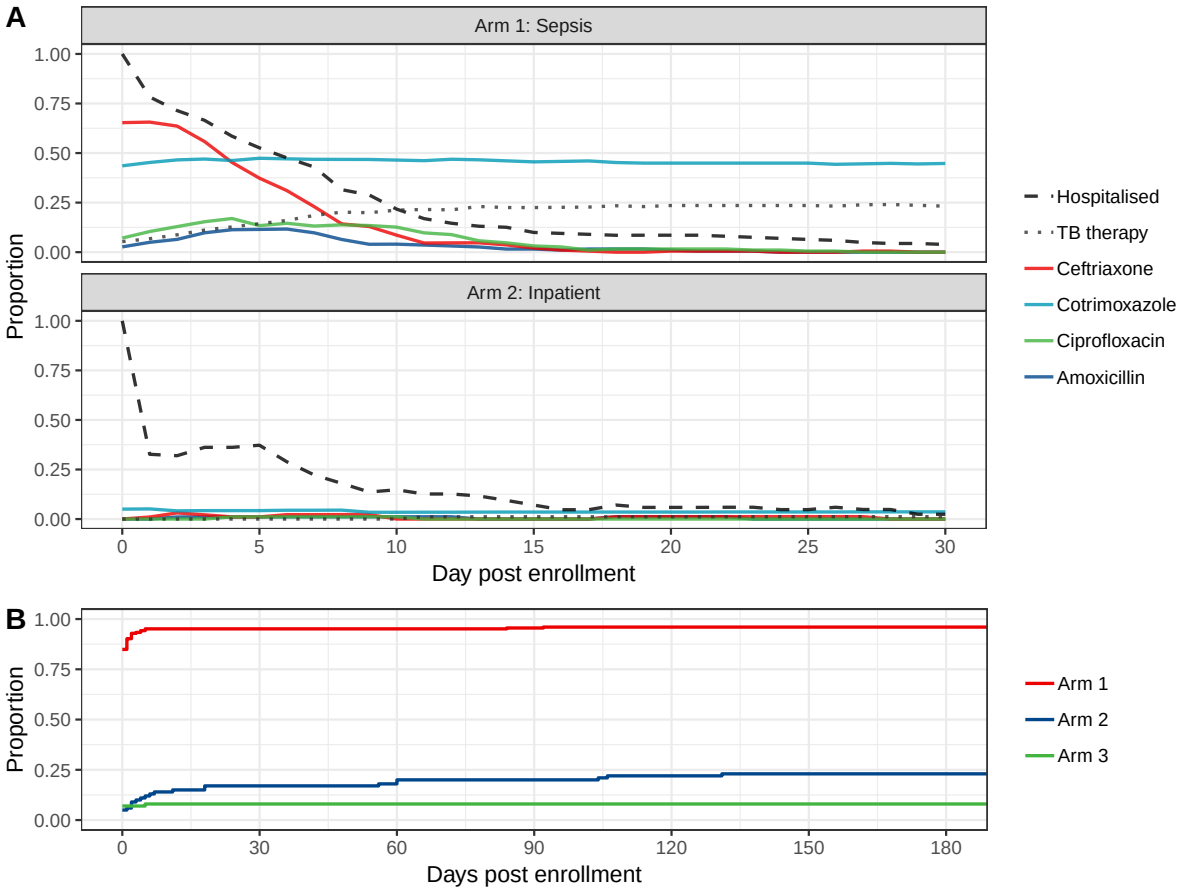

Figure 5.3: Hospital and antibacterial exposure of participants expressed as (A) proportion of Arm 1 and Arm 2 participant who are hospitalised and/or exposed to the most commonly received antibacterials on any given day and (B) cumulative proportion of participants who have been exposed to any antibacterial over the study period.

Table 5.2: Antimicrobial and hospital exposure stratified by arm

	Number exposed			Exposure (person-days)			Median (IQR) exposure length (days)		
Exposure	Arm 1	Arm 2	Arm 3	Arm 1	Arm 2	Arm 3	Arm 1	Arm 2	Arm 3
Total At Risk	225	100	100	33797	14336	21983	-	-	-
Exposures									
Hospitalised	225	100	1	1727	500	1	5 (2-10)	2 (2-7)	1 (1-1)
Cotrimoxazole	110	6	7	14447	549	1388	180 (27-190)	86 (6-177)	190 (183-206)
TB therapy	52	2	0	6843	291	0	178 (58-180)	146 (133-158)	-
Ceftriaxone	183	7	0	997	26	0	5 (3-7)	3 (2-4)	-
Ciprofloxacin	61	2	0	398	12	0	7 (5-7)	6 (6-6)	-
Amoxicillin	38	3	1	235	21	5	7 (5-7)	5 (5-8)	5 (5-5)
Metronidazole	24	2	0	148	10	0	6 (2-7)	5 (5-5)	-
Fluconazole	27	0	0	118	0	0	3 (2-5)	-	-
Aciclovir	2	0	0	47	0	0	24 (16-31)	-	-
Co-amoxiclav	10	2	0	40	12	0	5 (2-5)	6 (6-6)	-
Erythromycin	5	0	0	38	0	0	7 (5-11)	-	-
Doxycycline	7	0	0	34	0	0	3 (2-6)	-	-
Artesunate	11	0	0	25	0	0	2 (2-3)	-	-
LA	7	0	0	19	0	0	3 (2-3)	-	-
Streptomycin	2	0	0	16	0	0	8 (7-9)	-	-
Gentamicin	4	0	0	15	0	0	4 (3-5)	-	-
Amphotericin	2	0	0	8	0	0	4 (4-4)	-	-
Azithromycin	2	2	0	7	12	0	4 (3-4)	6 (6-6)	-
Penicillin	2	0	0	5	0	0	2 (2-3)	-	-
Flucloxacillin	2	0	0	5	0	0	2 (2-3)	-	-
Chloramphenicol	1	0	0	1	0	0	1 (1-1)	-	-
Quinine	1	0	0	1	0	0	1 (1-1)	-	-

Note:
TB = tuberculosis, LA =lumefantrine artemether. Median exposure length includes only those exposed. Total at risk shows the total number of participants and participant-days of follow up included in the study.

Table 5.3: ESBL carriage stratified by arm and visit

	Arm 1 (Sepsis)		Arm 2 (Inpatient)		Arm 3 (Community)	
Visit	n	Any ESBL	n	Any ESBL	n	Any ESBL
Day 0	222	109 (49%)	99	41 (41%)	99	28 (28%)
Day 7	162	127 (78%)	63	32 (51%)	-	-
Day 28	148	106 (72%)	71	37 (52%)	92	29 (32%)
Day 90	126	71 (56%)	60	29 (48%)	-	-
Day 180	127	61 (48%)	65	29 (45%)	83	24 (29%)

5.4.3 ESBL-E colonisation

ESBL-E colonisation prevalence as a function of time across the three arms of the study is shown in Table 5.3 and Figure 5.4. Baseline colonisation prevalence was high in all groups, and higher in hospitalised participants than community members: 49% (95% CI 42-56%) in Arm 1 participants, 41% (95% CI 32-52%) in Arm 2 and 28% (95% 20-38%) in Arm 3. Both hospitalised groups showed a rise in colonisation prevalence following admission, though this is much more marked in Arm 1 participants: by the day 7 visit 78% (95% CI 71-84%) of Arm 1 participants were colonised compared to 51% (38-64%) of Arm 2 participants. By the end of the study period the prevalence had fallen back to baseline levels in both groups.

In total, 723/1417 (51%) of samples grew at least one ESBL-E; 1032 organisms were grown from the 723 samples, with a median 1 (IQR [1-2]) ESBL-E per sample. The most commonly isolaed organism as identified by the API system was *E. coli* (n = 686) followed by *Klebsiella pneumoniae* (n = 245, Figure 5.5). Antimicrobial sensitivity testing was carried out on the first 694/1032 (67%) organisms; meropenam and amikacin sensitivity was near universal (680/694 [98%] and 679/694 [98%] of isolates respectively), but cotrimoxazole sensitivity very unusual (19/694 [3%] of isolates), with intermediate proportions of gentamicin (327/694 [47%]) and ciprofloxacin (237/694 [34%]) sensitivity. The antimicrobial to which the greatest proprtion of isolates were sensitive was chloramphenicol (462/694 [67%] of isolates).

5.4.4 Associations of ESBL colonisation

I then used logistic regression to explore associations of ESBL-E colonisation at baseline Of the 420 participants with an available enrollment stool culture result, 42% (178/420) cultured at least one ESBL-E. Univariable and multivariable associations of colonisation at enrollment are shown in 5.4. In univarible analysis HIV infection, ART and CPT are associated with ESBL-E colonisation, but this seems to be largely mediated by CPT as the HIV and CPT associations largely dissapear on multivariable modelling but the effect of CPT is still apparent (aOR 2.3 [95% CI 1.0 - 5.5]). Hospitalistion within the 4 weeks prior to admission was

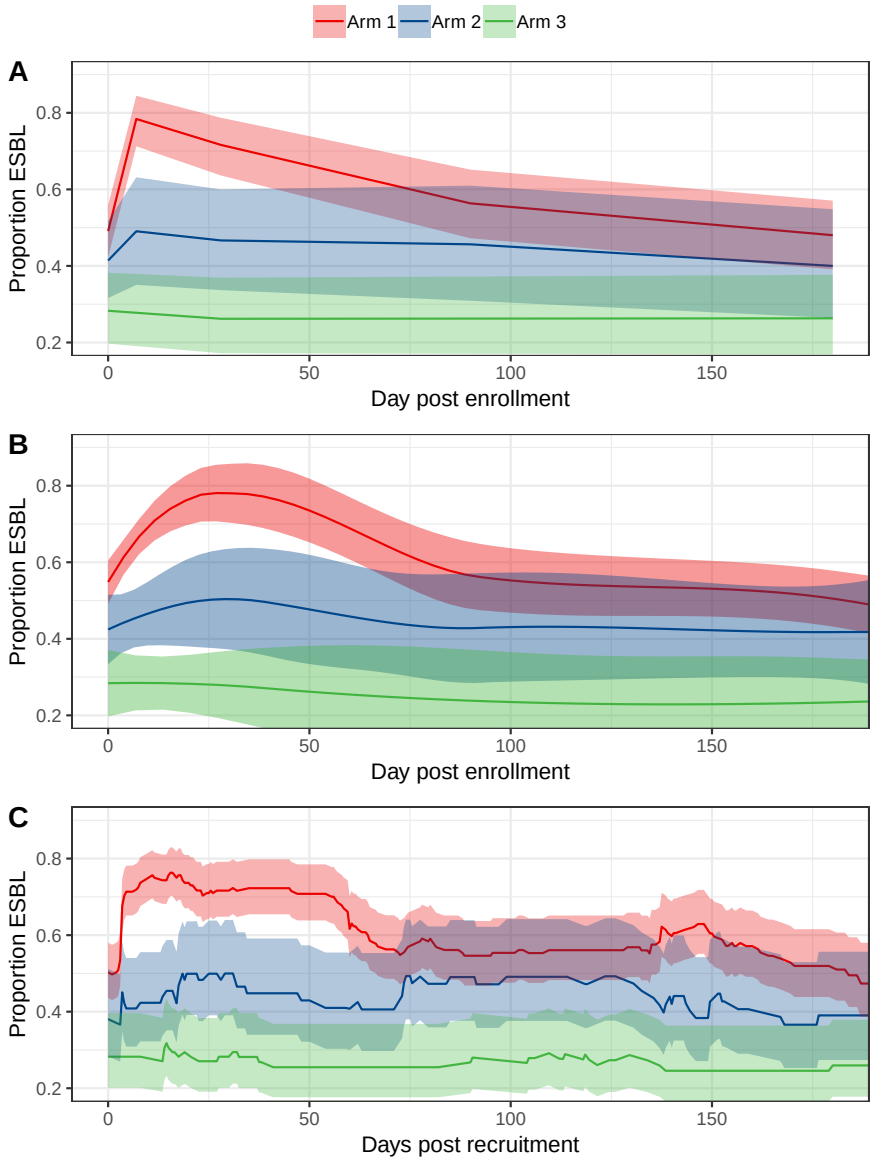

Figure 5.4: ESBL carriage prevalence as a function of time visualised in a number of different ways. In each case participants from Arm 2 are censored on first antimicrobial exposure and Arm 3 are censored on first antimicrobial exposure or hospitalisation. Top (A) prevalence at each visit plotted at ostensible visit time; however, the visits are in fact distributed in time themselves so the middle plot (B) is an attempt to show this by fitting a nonparametric smoothed LOESS regression line with a local linear regression. However the confidence intervals in this method are too narrow because they assume independence of the measurements, which are in fact clustered within patients. The bottom panel (C) is an estimate of the proportion of ESBL-colonised participants from the Aalen-Johansen estimate, which is a generalisation of the Kaplan-Meier curve. This takes into account the nonindependence of the measurements, but does not take into account the interval-censored nature of the data, and transitions to and from the ESBL colonised state are hence assumed to happen halfway between measurements. The best estimate of state occupancy to account for all these difficulties requires the fitting of a Markov model: see chapter xx.

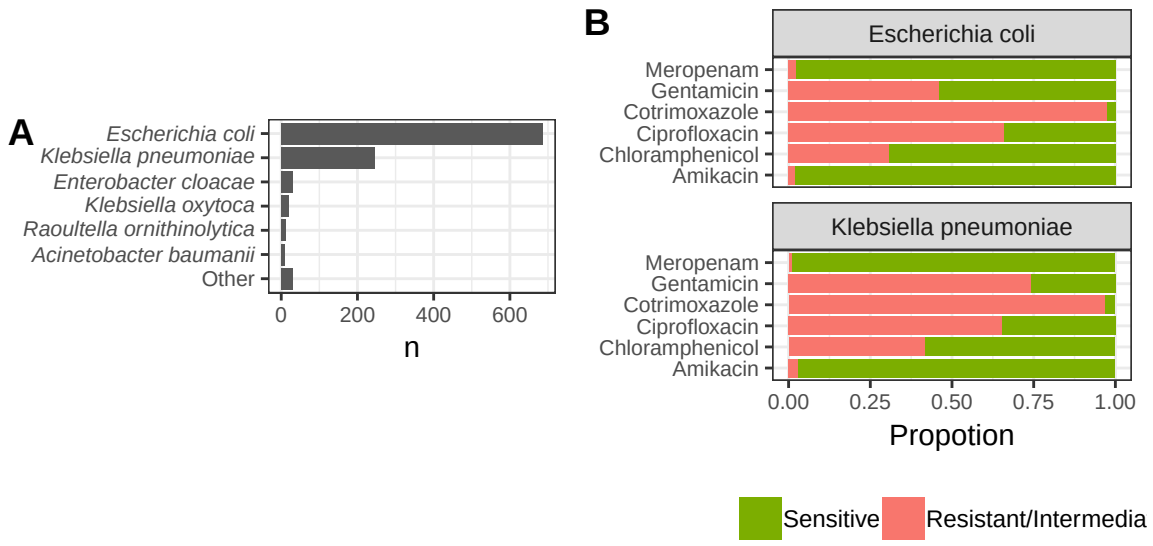

Figure 5.5: Species (A) and antimicrobial sensitivities (B) of cultured ESBL-E

strongly associated with ESBL-E colonisation on multivariable modelling, though with wide confidence intervals (aOR 5.9 [95% CI 1.8-27.0]), perhaps expected as it is a rare baseline exposure. Antimicrobial exposure was not, but with confidence intervals that contained a clinially relevant effect size (aOR 1.3 [95% 0.7 - 2.6]). ESBL-E colonisation was more likely with more adults in the household (aOR 1.2 [95% CI 1.0-1.4] per exta adult) , with use of an unprotected water source (aOR 3.0 [95% CI 1.1 - 8.8]) and in the rainy season (aOR 2.2 [95% CI 1.4-3.4]); a constellation of variables that are consistent with a significant role of community faecal-oral transmission in the spread of ESBL-E.

To explore associations of acquisition of ESBL-E by the day 28 visit, I analysed only those participants who had no detectiable ESBL-E at basline, and an available follow up samples at 28 days +/- 14 days. These numbered 150 participants: 64 Arm 1, 37 Arm 2 and 49 Arm 3 participants, and 49% (73/77) of them had a detectable ESBL-E at day 28. Bivariable associations of ESBL-E aquistion with animicrobial and hospital exposures are shown in Figure 5.6A, stratified by the length of exposure; all antibacterials (including TB therapy) showed an association with ESBL-E acquisition, with a suggestion of a dose-response effect, but confidence intervals were large in many cases. Antimalarials did not show this effect though here undcertainty in the estimates precludes drawing any firm conclusions, as it does for antifungals. These relationships are very likely confounded, so shold be regarded with extreme caution; however, due to a small dataset size and collinearity, logistic regression modelling of ESBL-E acquisition (Figure 5.6B) produces such uncertain parameter estimates that no conclusions can be drawn. A better modelling strategy using continuous time Markov models was used to understand the drivers of ESBL-E carriage; this analysis is presented in

Table 5.4: Univariable and multivariable associations of ESBL colonisation at enrollment

	Univariable		Multivariable	
Variable	OR (95% CI)	p-value	aOR (95% CI)	p-value
Demographics				
Age (per year)	1.00 (0.99-1.02)	0.709	1.00 (0.98-1.02)	0.898
Male sex (vs female)	1.23 (0.84-1.82)	0.287	1.42 (0.93-2.19)	0.106
Study Arm				
Arm 2 (vs 1)	0.73 (0.45-1.18)	0.203	1.57 (0.84-2.96)	0.157
Arm 3 (vs 1)	**0.41 (0.24-0.68)**	**0.001**	0.91 (0.45-1.84)	0.801
HIV status				
HIV+ (vs HIV-)	**1.68 (1.09-2.59)**	**0.018**	1.16 (0.46-2.84)	0.750
HIV unknown (vs HIV-)	0.71 (0.40-1.24)	0.229	1.09 (0.55-2.18)	0.798
CPT (vs none)	**2.46 (1.58-3.86)**	**<0.001**	2.29 (0.98-5.54)	0.060
ART (vs none)	**1.99 (1.32-3.00)**	**0.001**	1.06 (0.35-3.17)	0.918
Exposures last month				
Hospitalisation	**7.87 (2.57-34.22)**	**0.001**	**5.90 (1.78-26.94)**	**0.008**
Antibiotics*	**2.14 (1.27-3.67)**	**0.005**	1.34 (0.71-2.57)	0.368
Household size				
Children (per 1)	1.00 (0.87-1.14)	0.979	0.98 (0.84-1.14)	0.793
Adults (per 1)	1.14 (0.99-1.31)	0.064	**1.19 (1.02-1.40)**	**0.026**
Keep animals (vs not)	1.33 (0.88-2.03)	0.176	1.16 (0.73-1.85)	0.527
WaSH behaviour				
Flushing toilet (vs not)	1.38 (0.55-3.44)	0.481	0.94 (0.34-2.55)	0.908
Unprotected water source	2.43 (0.96-6.64)	0.068	**2.98 (1.08-8.78)**	**0.039**
Treat water (vs not)	1.16 (0.50-2.66)	0.725	0.94 (0.37-2.34)	0.900
Season				
Rainy season (vs. dry)	**2.05 (1.38-3.06)**	**<0.001**	**2.17 (1.38-3.44)**	**0.001**

Note:
CPT = Cotrimoxazole preventative therapy, ART = antiretroviral therapy, WaSH = Water, sanitation and hygiene. Entries in bold are those for which 95\% confidence intervals do not cross 1.
* Antibiotics includes TB therapy but excludes CPT.

Chapter xx.

5.5 Discussion

In this chapter, I have presented the data which begins to address the second aim of this thesis: to describe, and identify determinents of, ESBL-E acquisition and carriage in Malawian adults as they pass through the hospital. It is possible to draw several conclusions from these data. First, community ESBL-E carriage is common, and the prevalence is high compared to high income settings, comparable to many community studies from elsewhere in sSA and other high-prevalence settings such as India. The baseline community carriage prevalence of 28% is considerably higher than the 4-7% seen in Europe[[1];[2];Ny2017;Valverde2004] and

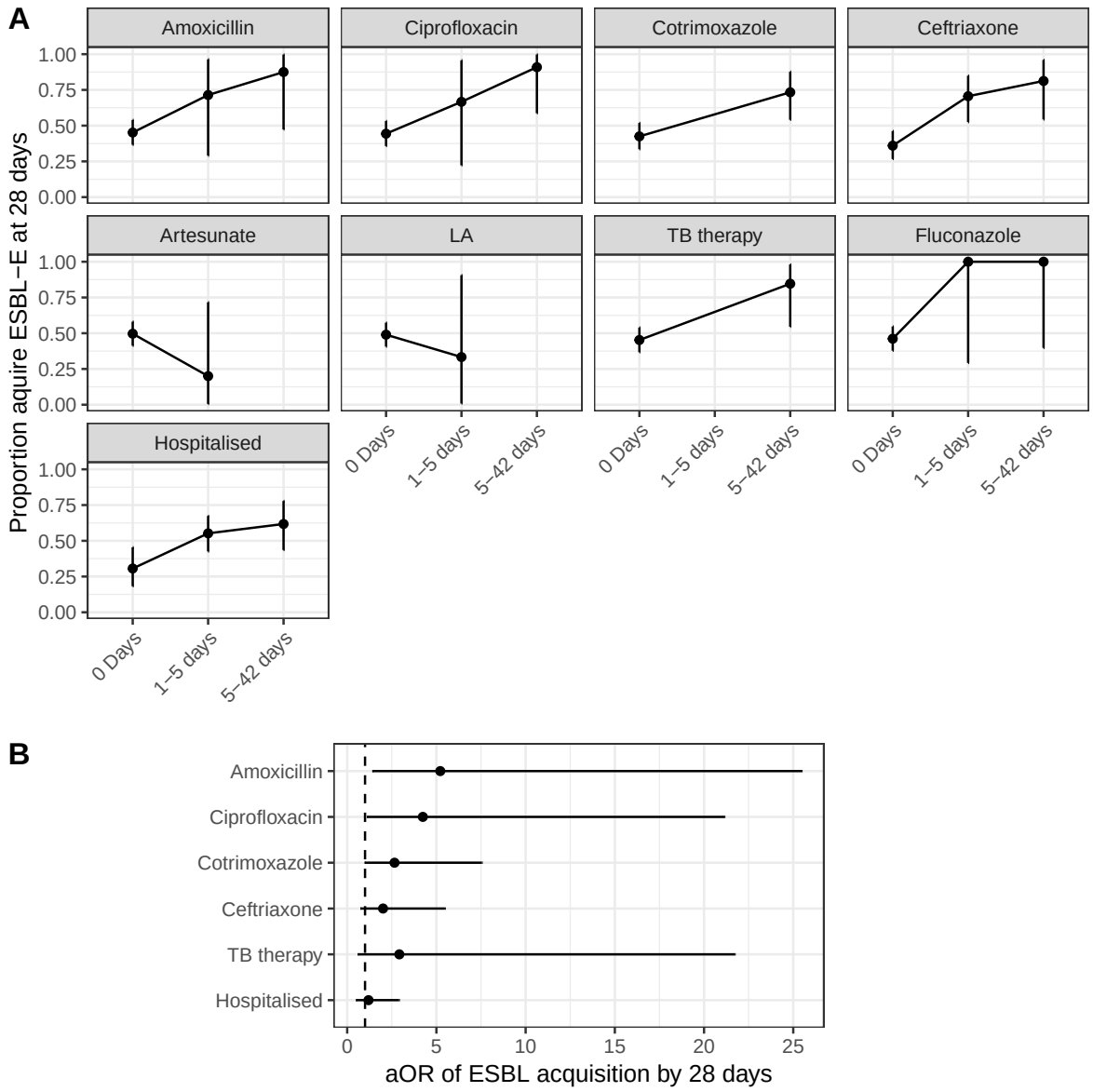

Figure 5.6: Association of ESBL-E acquisition by 28 days. Bivariable (A) and multivariable (B) associations of antimicrobial and hospital exposure with acquisition of ESBL-E by 28 days. A: These plots show the proportion of participants who have no detectable ESBL-E baseline but who do at 28 days, as a function of various exposures. All antibacterials and hospitalisation show an association between exposure and ESBL-E acquisition, with a suggestion of a dose-response relationship, though confidence intervals are wide in many cases. Antimalarials show no apparent relationship though, as with fluconazole, the wide confidence intervals make it difficult to draw any conclusions. The results from logistic regression to predict ESBL-E acquisition are shown in (B); colinearity and small dataset size means that confidence intervals are so large as to make the model useless. A better approach to modelling ESBL-E acquisition, Markov modelling, is shown in Chapter xx.

comparable to the sSA pooled community prevalence estimate presented in Chapter 1 of 18% (95% CI [11-27%]).

Secondly, the associations of baseline ESBL-E carriage give insight into the drivers of ESBL-E transmission in the Malawian setting. Household crowding, and use of unprotected water sources and are associated with ESBL-E colonisation, suggesting that both household transmission and environmental reservoirs play a role in the spread of ESBL-E in Blantyre. The number of adults in the household, rather than the number of children, was associated with ESBL-E carriage in the adults in this study, suggesting that within the household adult to adult transmission is a more important route than child to adult transmission. This could be for a number of reasons - if the ESBL-E prevalence were low in children, for example. Though children were not sampled in this study and so the data here can not address that hypothesis, data from other studies suggest this is unlikely: community prevalence in children ranged from 10-59% in four studies in the Central African Republic[3], Senegal[4] and Tanzania[[5];Moremi2017], and is hence comparable to the adult community prevalence seen in this study. Behavioural factors, or a lower bacilliary burden in children could also account for the associations seen here.I also demonstrate a seasonality to ESBL-E colonisation. This is again consistent with environmental spread of ESBL-E - faecal oral transmission of bacteria could be more likely in the rainy season - but changes in behaviours during the rains could also contribute.

5.5.1 Limitations

5.6 Conclusions and further work

Chapter 6

Whole genome sequencing of ESBL *E. coli* carriage isolates

Placeholder

6.1 Chapter overview

6.2 Methods

6.2.1 Bioinformatic pipeline

6.2.2 Global *E. coli* collection

6.2.3 Statistical analysis

6.3 Results

6.3.1 Samples and quality control

6.3.2 Phylogroup, MLST and core genome phylogeny of study isolates

6.3.3 Study isolates in a global context

6.3.4 Antimicrobial resistance determinants

6.3.4.1 β-lactam resistance

6.3.4.2 Quinolone resistance

6.3.4.3 Aminoglycoside resistance

6.3.4.4 Chloramphenicol, co-trimoxazole, tetracycline and other resistance determinants

6.3.4.5 Clustering and lineage association of AMR determinants

6.3.5 Plasmid replicons

6.3.6 Testing metadata associations: SNP distance, hierBAPS sequence clusters and ESBL-clusters

6.3.6.1 Hierarchical BAPS clustering of core gene pseudosequences

6.3.6.2 ESBL-clusters

6.3.6.3 Assessing for healthcare-associated lineages

6.3.6.4 Assessing for within-patient conservation of lineage or MGE

6.4 Discussion

Chapter 7

Gut mucosal carriage of ESBL-E in Blantyre, Malawi

References

1 McNulty CAM, Lecky DM, Xu-McCrae L *et al.* CTX-M ESBL-producing Enterobacteriaceae: estimated prevalence in adults in England in 2014. *The Journal of antimicrobial chemotherapy* 2018;**73**:1368–88. doi:10.1093/jac/dky007

2 Wielders C, Hoek A van, Hengeveld P *et al.* Extended-spectrum β-lactamase- and pAmpC-producing Enterobacteriaceae among the general population in a livestock-dense area. *Clinical Microbiology and Infection* 2017;**23**:120.e1–8. doi:10.1016/J.CMI.2016.10.013

3 Farra A, Frank T, Tondeur L *et al.* High rate of faecal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae in healthy children in Bangui, Central African Republic. *Clin Microbiol Infect* 2016;**22**:891.e1–4. doi:10.1016/j.cmi.2016.07.001

4 Ruppe E, Woerther PL, Diop A *et al.* Carriage of CTX-M-15-producing Escherichia coli isolates among children living in a remote village in Senegal. *Antimicrob Agents Chemother* 2009;**53**:3135–7. doi:10.1128/aac.00139-09

5 Tellevik MG, Blomberg B, Kommedal O *et al.* High Prevalence of Faecal Carriage of ESBL-Producing Enterobacteriaceae among Children in Dar es Salaam, Tanzania. *PLoS One* 2016;**11**:e0168024. doi:10.1371/journal.pone.0168024