Developing an Antimicrobial Strategy for Sepsis in Malawi

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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).

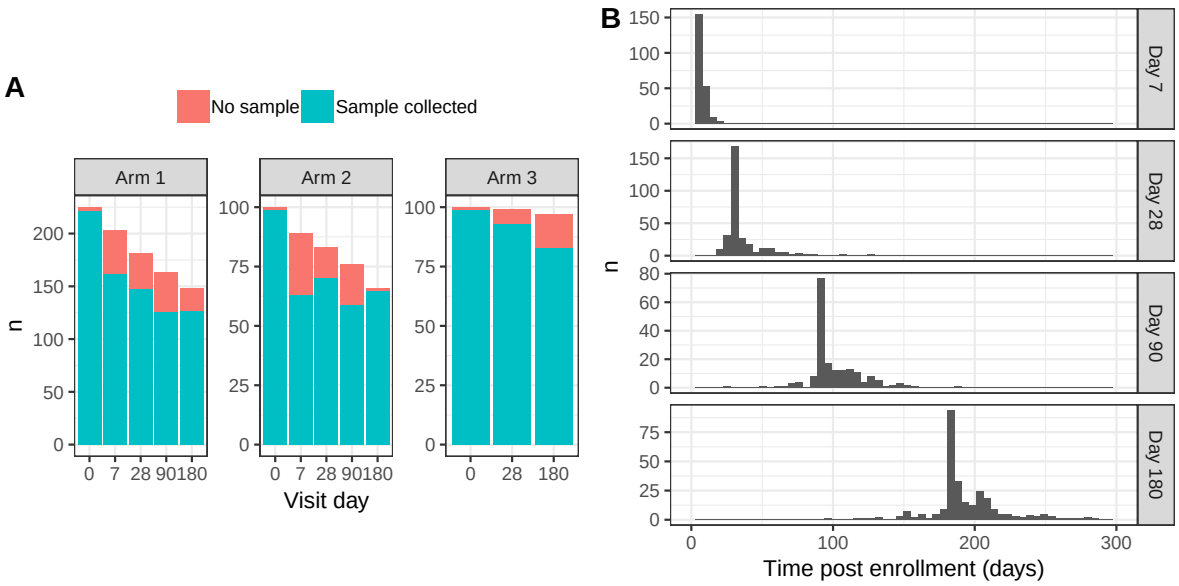

Figure 5.1: A: Missing stool samples stratified by arm and visit. Bar height at a given visit represents the number of eligible participants, coloured by successful sample collection (blue) or failure to collect a sample (red). B: Distribution of actual day of sample collection for ostensible day 7,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

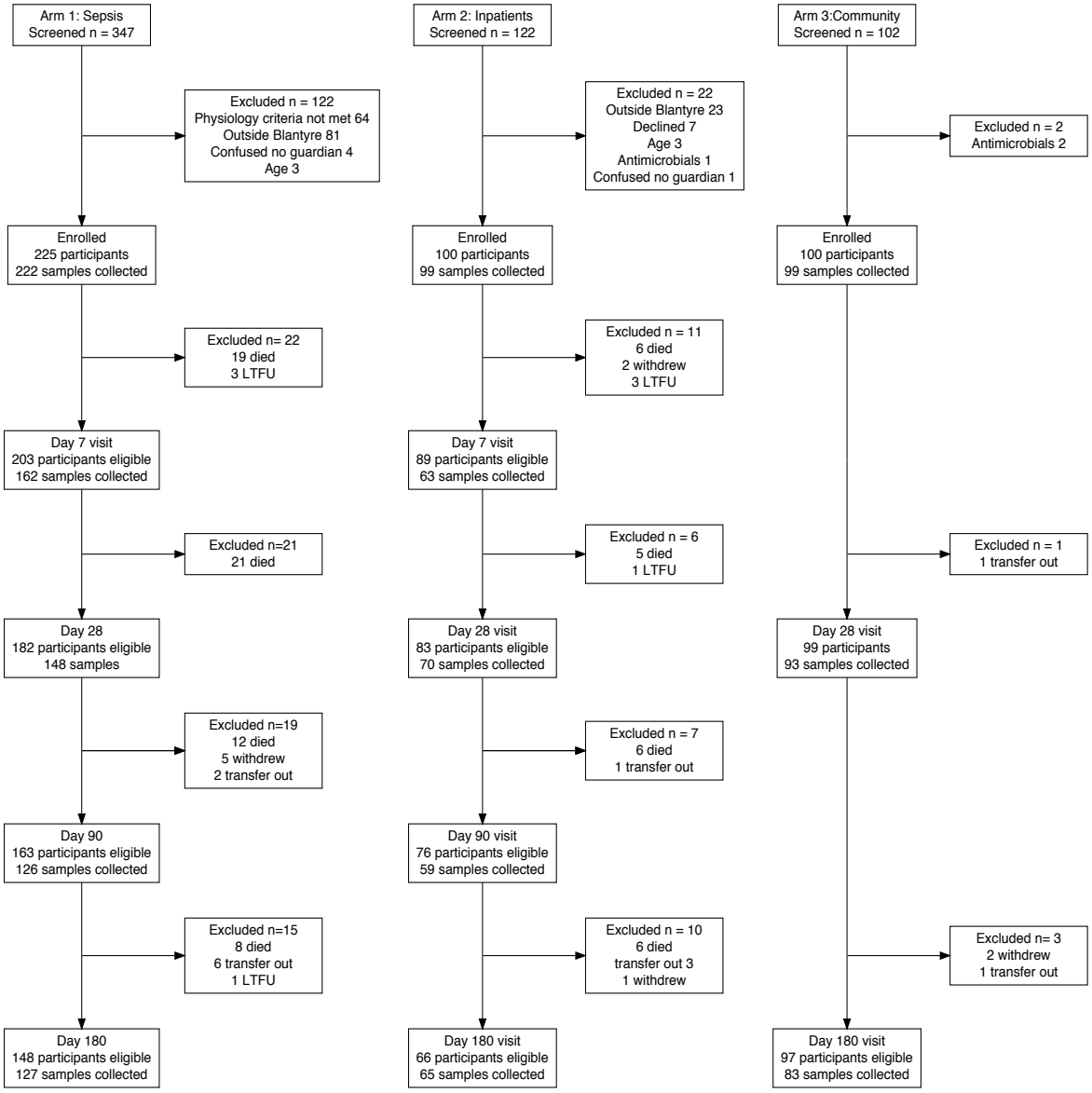

Figure 5.2: Study recruitment and follow up. At each time point *eligible participants* refers to participants who are known to be alive and have not withdrawn from the study by that time point, and *samples collected* refers to patients from whom a stool sample was sucessfully collected for that visit.

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 Figures 5.3 and 5.4 and Table 5.2. Antimicrobial-unexposed inpatients (Arm 2 participants) had a shorter length of hospital stay than participants with sepsis (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, most commonly within 30 days following enrollment (Figure 5.4), 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 on admission. 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).

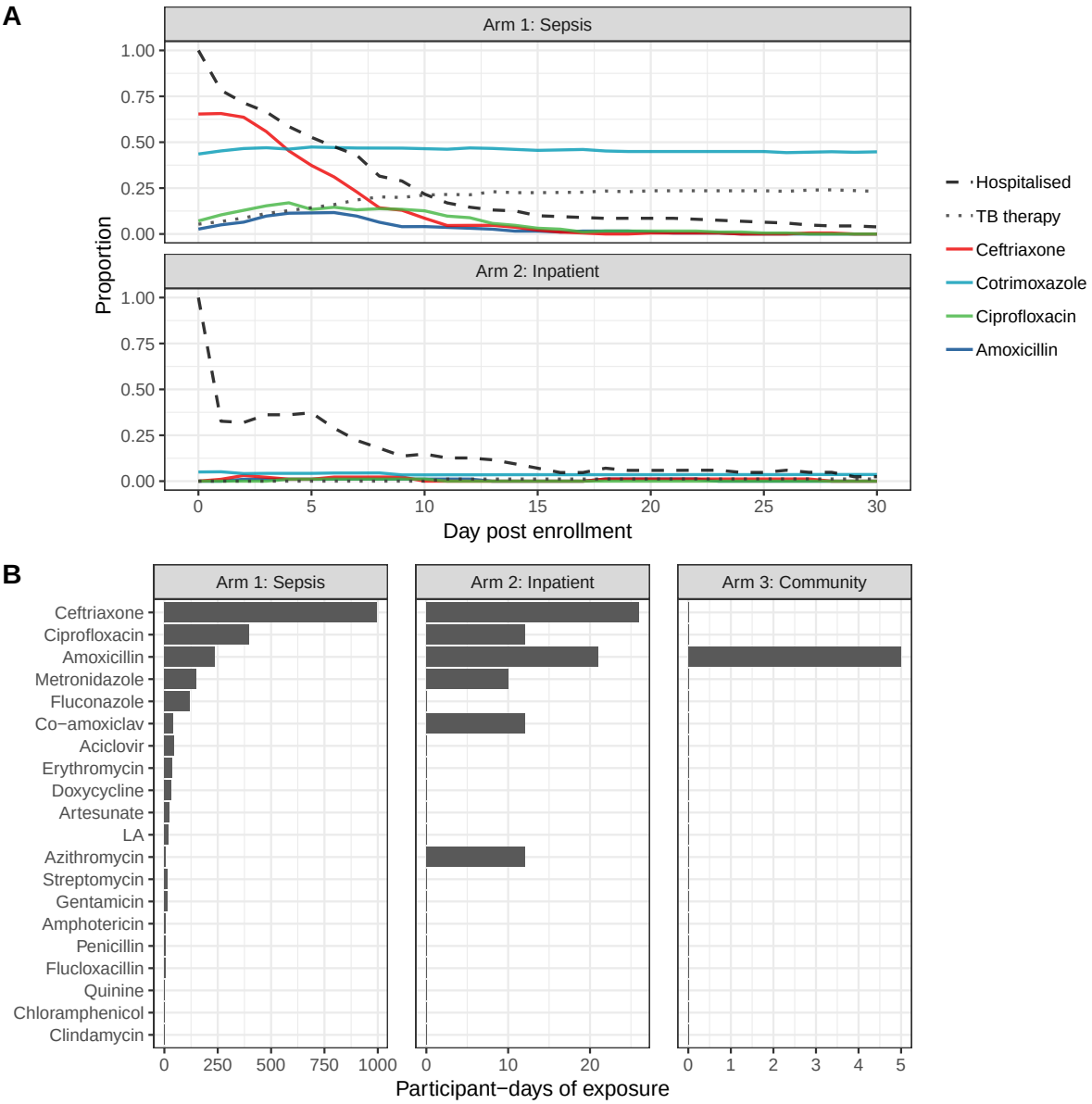

Figure 5.3: Hospital and antibacterial exposure of participants expressed as (A) proportion of participants in the sepsis (arm 1) and inpatient antimicrobial-unexposed (arm 2) groups who are hospitalised and exposed to the most commonly received antibacterials, as a function of time and (B) participant-days of exposure to the most commonly received antibacterials (excluding TB therapy and co-trimoxazole) across the three arms of the study over the whole 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.

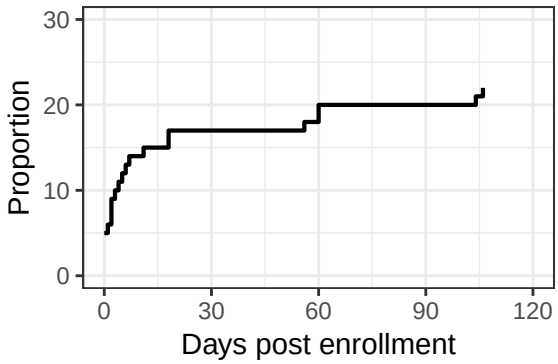

Figure 5.4: Cumulative number of arm 2 particpants exposed to antibacterials (including CPT and TB therapy) as a function of time.

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 5.5. Baseline colonisation prevalence was high in all groups, and higher in hospitalised participants than community members: 49% (95% CI 42-56%) in participants with sepsis, 41% (95% CI 32-52%) in baseline antimicrobial-unexposed inpatients and 28% (95% 20-38%) in community members. In crude analyses, both hospitalised groups show 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.

5.4.4 Determinents of baseline ESBL colonisation

5.4.5 Determinents of d28 ESBL-E acquisition

5.5 Discussion

5.5.1 Limitations

5.6 Conclusions and further work

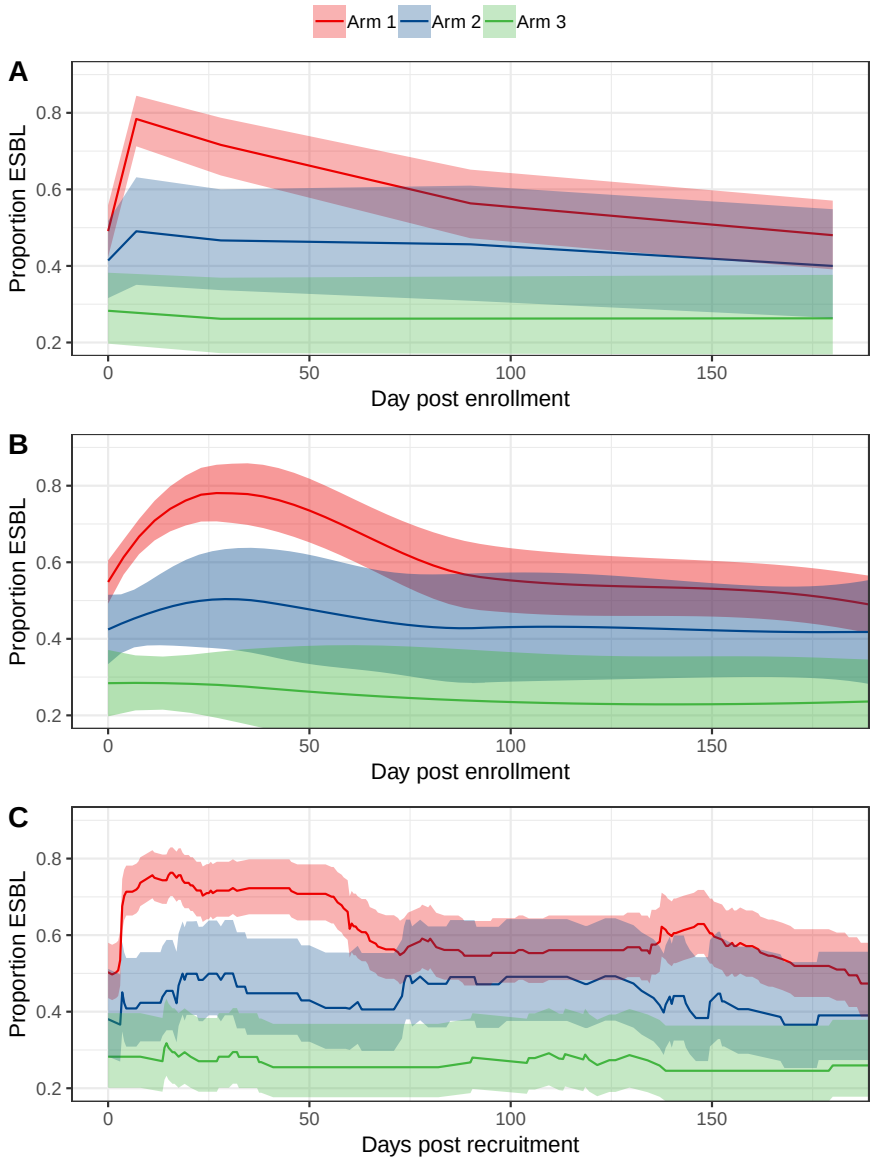

Figure 5.5: ESBL carriage prevalence as a function of time visualised in a number of different ways. In each case participants from Arm 2 are censored on antimicrobial exposure and Arm 3 are censored on antimicrobial exposure or hospitalisation. Top (A) prevalence at each visit plotted at ostensible 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 indepence 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 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.

Chapter 6

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

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Chapter 7

Gut mucosal carriage of ESBL-E in Blantyre, Malawi

References