

# Developing an Antimicrobial Strategy for Sepsis in Malawi

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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

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# Preface

Placeholder



# Chapter 1

## Introduction

Placeholder



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## Chapter 2

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## Chapter 4

# Sepsis in Blantyre, Malawi

### 4.1 Chapter overview

### 4.2 Aims and Methods

The clinical and laboratory methods of the clinical study are described in Chapter 2, Methods; a further overview of chapter aims and description of the statistical analysis used is given here. The aims of this chapter are twofold. First, a description of the presentation, aetiology, therapies delivered, and outcomes of sepsis in Blantyre; that is, the demographics and health seeking behaviour of patients presenting with sepsis, along with description of microbiologic causes identified, therapies delivered (antimicrobials and intravenous fluids), outcomes to 180 days in terms of mortality presented at 28, 90 and 180 days, and health related quality of life. Second, an analysis to identify associations of mortality and to develop models to attempt to understand the causal effect of interventions delivered to patients presenting with sepsis.

For the first aim - description of sepsis in Blantyre - patient demographics, health seeking behaviour, symptoms and admission physiology are described as medians and interquartile ranges for continuous variables or proportions for categorical variables, and Kruskal-Wallis or Fisher's exact tests used to compare between groups. Aetiology is presented as simple proportions, stratified by HIV status and co-infections visualised as Venn diagrams and UpSet plots using *eulerr* and *UpSetR* packages, respectively, in R. Mortality is presented as simple proportions at 28, 90 and 180 days, and Kaplan-Meier estimation of the survival function generated using the *survival* package in R; both of these estimates are presented aggregated and stratified by HIV status, with log-rank test used to test the hypothesis that HIV-infected and uninfected survival functions differ. Morbidity was assessed as health related quality of

life (HRQoL) using the EQ-5D-3L questionnaire, which assesses HRQoL across five domains (anxiety, pain, self care, usual activities, walking) with participants describing their problems across the domains on a 3 point ordinal scale: no problems, moderate or extreme. So-called tariff sets are used to convert these scores to an overall utility score, which compares the health state compared to perfect health: a utility score of one indicates that a health state is the same as usual health and zero represents death, but scores of below zero (states worse than death) are possible. Tariff sets are country specific, and no Malawian tariff sets are available, so a Zimbabwean tariff set was used. The *eq5d* package in R was used to convert health states into utility scores. HRQoL was measured at baseline, and the 1,4,12 and 24 week visits, and is presented as proportion of participants reporting at least moderate impairment in each domain (with exact binomial confidence intervals) at each time point, as well as boxplots of utility score. Utility scores of participants with sepsis was compared to community controls using t-tests.

The second aim - models to understand the causal effect of interventions delivered to patients with sepsis - presents conceptual and technical difficulties. There are a number of standard modelling approaches in the biomedical literature when putative associations between predictor variables and mortality are to be identified. The usual approach consists of selecting variables using some criteria as variables to be included as predictors in a regression model, and identified associations are interpreted as the independent effect of the included variables. There are two common problems with this approach. Firstly, commonly used variable selection strategies have the possibility of introducing significant bias, if they use associations within the data to guide inclusion of variables e.g. bivariate associations or stepwise variable inclusion using statistical significance (or other) thresholds. This is because the statistics used to test the parameters (and generate confidence intervals around effect sizes etc.) are based on an assumption that a single hypothesis is being tested, an assumption which is violated by the stepwise model building process. It can be shown that standard errors are too small, that p-values are biased towards zero and parameter estimates biased away from zero [Harrell book]. But selecting variables to be included in a regression model is a difficult problem with no consensus on an ideal solution; *a priori* selection of variables for theoretical reasons is likely ideal, but this becomes difficult when there are a large number of potentially important predictors. This is because including more predictor variables - though it may decrease bias in the estimates of the model - increases the variance of the predicted values, the so-called bias-variance trade off. Dimensionality reduction techniques (such as principal components analysis) or shrinkage methods (lasso or ridge regression) have been suggested as alternative predictor variable selection techniques.

Secondly, even if a regression model is correctly specified in terms of predictor variables, correct interpretation of predictor effects is often difficult or impossible without a clear hypothesised

causal structure. For example, consider a hypothesised causal structure of death in sepsis in Figure 4.1, which I express as a directed acyclic graph (DAG); nodes represent collections of variables which theoretically specify host status (age, sex, immune status including HIV status and CD4 cell count), infection type (e.g. causative pathogen, site), disease severity (e.g. physiological variables quantifying shock, hypoxia etc.), therapies administered, and outcome. Arrows (called edges in the DAG framework) show causality: host status influences infection (e.g. TB is more common in HIV) and severity (patients with advanced HIV may have more severe infection), for example, and therapies administered is likely to be influenced by disease severity (perhaps sicker patients receive antimicrobials more quickly), host status (clinicians are likely to administer different therapies to HIV-infected patients), and infection type. A standard analysis of sepsis would construct a predictive multivariable model for death by including factors which the analyst felt likely to be associated with mortality, which would usually include HIV status, CD4 cell count, physiologic variables (such as presence of shock) and infection variables (e.g. presence of bloodstream infection [BSI]). The effects of the predictor variables are often then interpreted as the independent effect of the included predictors, after controlling for all others; however, this may not be the case. For example, severity is at least in part a mediator of the effect of HIV on outcome, so the interpretation of the coefficient of HIV in such a model is the residual effect of HIV once disease severity is accounted for. It is likely that there are direct effects of host and infection factors on outcome (dotted edges in Figure 4.1, not least because measured variables in a study are unlikely to wholly quantify disease severity, but if not then controlling for disease severity will completely remove the effect of HIV status on mortality, which may not be the analysts intention, or interpretation of parameters. This has been called the “Table 2 fallacy.” It is important therefore to clearly define the effect that is being sought from an analysis (e.g. the effect of HIV status on mortality) and to ascertain which factors need to be controlled for based on this. It may be that a number of different models are necessary to estimate parameters of interest, if more than one parameter is of interest. The causal inference framework provides tools to do this using DAGs, and the *dagitty* package in R automates this framework so, when provided with a DAG, it can output the variables that must be conditioned upon to estimate the causal effect of an exposure on an outcome.

In this chapter, therefore, the aim of the analysis is to provide an estimate of the effect of treatments administered on mortality; the class of antimicrobial administered as well as the time-to-antimicrobial for different classes, and the volumes of intravenous fluid administered. this will inform the overarching aim of the thesis - to develop novel antimicrobial strategies for sepsis in sSA to improve outcomes. This will require correcting for (or conditioning on) host, infection, and severity variables (assuming a direct effect of infection and host on outcome). These variables (18 in total) have been selected *a priori* and are shown in

Table ?? in the results section. I chose to use the method of factor analysis of mixed data (FAMD) from the *FactoMineR* package in R to perform dimensionality reduction on these variables. This technique uses principal component analysis (PCA) for continuous variables and multiple correspondence analysis (MCA) to generate a new orthogonal coordinate system which maximises explained variance in each FAMD axis. As well as reducing the dimensionality of the dataset, this technique has the advantage of ensuring an orthogonal coordinate system; the untransformed variables are correlated (e.g. shocked patients are likely to have elevated lactate, low blood pressure, low bicarbonate, and high heart rate) and so parameter estimates become very large when entered into a regression model. This is the problem of collinearity.

Non-normally distributed variables (temperature, white cell count, urea, creatinine, lactate) were identified on kernel density plots and transformed with a natural logarithm prior to FAMD, and all variables mean centered and scaled using standard deviation; two variables (oxygen saturation and Glasgow coma score [GCS]) remained nonnormal following logarithmic transformation and were recoded as binary variables with a cut off of less than 92% (for oxygen saturation) and less than 15 (for GCS). Because CD4 count was unavailable for HIV-uninfected participants, CD4 count was recoded as a binary dummy variable to be equal to 1 for HIV-infected participants with a CD4 cell count less than 100 cells  $\mu\text{L}^{-1}$  and 0 for all other participants. The first three FAMD dimensions were used as predictors of mortality in a logistic regression model, which also included diagnosis (TB, BSI, meningitis, malaria) and treatment (whether the participant was administered antibacterials, antimycobacterial therapy, antifungals or antimalarials). Diagnostic variables were not transformed as they are largely orthogonal (i.e. it is not possible to reduce their dimensionality to any great extent without losing information). Volume of intravenous fluid administered over 6 hours was included as a linear continuous predictor in this model.

Because of nonidentifiability of the model under a maximum likelihood framework, Bayesian regression using the *brms* package in R (a front end to the Stan probabilistic programming language) was used. No patients with malaria died, which meant that it is not possible for standard (maximum likelihood) models to estimate parameters for the effect of malaria mortality. Excluding this variable could result in biased estimates, but it is possible to fit the model in a Bayesian framework by specifying weakly informative priors on the parameters. In the broadest sense, we use our knowledge (that adults do die of malaria) to set priors that pull the parameter estimates to an identifiable value. Student's  $t$  distribution centred on 0 with three degrees of freedom and a scale of 2.5 were used, following Gelman et al. Four Markov-chain Monte-Carlo (MCMC) chains each with 1000 iterations and a burn-in of 500 iterations were used with default *brms* settings. Convergence was assessed using traceplots and assessing for autocorrelation using the Gelman-Rubin diagnostic ( $\hat{R}$ ) with a target of  $\hat{R} < 1.1$ ). Parameter estimates were calculated from the posterior using medians and 95%



confidence intervals.

Missing data were imputed using multiple imputation of chained equations using default settings in the *mice* package in R, with each missing variable predicted by all other missing variables, to produce 10 imputed datasets. Models were fit using *brms* and then pooled parameter values calculated by taking medians and 95% confidence intervals of pooled posterior parameter estimates from all imputed datasets.

Finally, the relationship between time-to-antimicrobials and mortality was assessed, initially in bivariate associations using nonparametric locally estimates scatterpoly smoothing (LOESS) regression which performs a rolling linear regression and estimates the probability of death by 28 days as a function of the predictor variables. Only for antibacterials were there sufficient data to construct regression models which used time to antibacterial therapy (mean-centred and scaled as before) as a predictor for death by 28 days, alongside the first three FAMD dimensions. In view of possible nonlinear relationship between time to therapy and death apparent in the bivariate plots both linear and second-order polynomial models were fit, as before. Coefficient estimates are presented, but because interpretation of polynomial coefficients is challenging, predicted probability plots with 95% confidence intervals with the levels of the other covariates set to their mean values were plotted, using all the posterior draws to generate the median prediction and 95% confidence intervals.

## 4.3 Results

### 4.3.1 Study population

Figure 4.2 shows flow through the study. 225 participants were recruited in 20 months between 19th February 2017 and 2nd October 2018. Participants were recruited, in general, soon after arrival in hospital, a median (IQR) of 1.5 (0.8-2.6) hours after fist attendance. In total, 4 participants (2%) were lost to follow up over the 180-day study period; 5 participants (2%) withdrew; and 7 participants (3%) transferred out of the study area before 180 days. Four of the five participants who withdrew gave a reason for their wish to withdraw, all that they no longer wished the inconvenience of being involved in the study. 15/225 (7%) participants had their final study visit before 180 days, and so were not included in the 180-day outcome analysis.

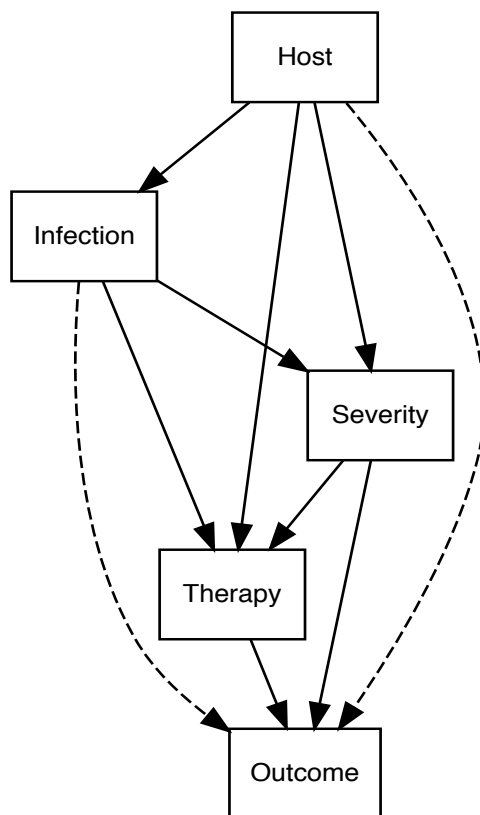


Figure 4.1: Hypothesised causal structure of death in sepsis. Host factors (e.g. age, sex, immune status) influence the type of infection; disseminated TB is more common in HIV, for example. Severity (variables quantifying e.g. shock or respiratory failure) is influenced by infection type and host factors. Therapy encodes which antimicrobials were administered and rapidity of administration of antimicrobials, and is influenced by disease severity (sicker patients may be given different therapies), host factors (HIV status may influence treatment) and the infection type (for example, malaria rapid diagnostic tests influencing rapidity of malaria treatment). Dotted edges from host and infection to outcome are because it is not clear *a priori* whether the effect of infection and host factors are entirely mediated by disease severity: in fact, even if this were the case in a theoretical sense, the available severity variables are unlikely to completely account for the causative effect of infection type on mortality and so conditioning on all available severity variables is likely to leave some residual causative effect of infection type. See text for further discussion

### 4.3.2 Symptoms and health-seeking behaviour

Table 4.1 shows the baseline characteristics of the recruited participants. They were young (median [IQR] age 36 [28-44]) and predominantly HIV-infected. Of those who were HIV-infected, the majority (117/143 [82%]) were on ART, almost exclusively the Malawian first-line regimen of efavirenz, lamivudine and tenofovir, and 88/117 (75%) had been taking ART for more than three months. Figure 4.3 shows the presenting symptoms of the participants. Almost all (221/225 [98%] of participants) experienced subjective fever. Participants had been unwell for some time, a median (IQR) of 7 (3-14) days; 32/225 (14%) of participants had been unwell for more than 4 weeks. 18/225 (8%) of participants had been admitted to hospital within the last 4 weeks. Over half (123/225 [55%]) of participants had sought care for their current illness (Table 4.2), most commonly (101/123 [82%] of participants) at a government health centre, a median (IQR) of 2 (1-6) days previously. 60/225 (27%) of all participants had received an antimicrobial for their current illness: 7/60 (12%) of all prehospital antimicrobials were antimalarials, the remainder antibacterial, most commonly co-trimoxazole or ciprofloxacin. Prehospital intravenous or intramuscular antimicrobials were administered in 16/60 (27%) participants receiving antimicrobials: ceftriaxone (n=6), benzylpenicillin (n=4), gentamicin (n=3) and artesunate (n=3).

Table 4.1: Participant Characteristics

Variable	Value
<b>Demographics</b>	
Age (years)	36 (28-44)
Male sex	114/225 (51%)
<b>HIV/TB status</b>	
HIV Reactive	143/225 (64%)
HIV Non Reactive	70/225 (31%)
HIV Unknown	12/225 (5%)
Ever treated for TB	37/225 (16%)
Of those, current TB treatment	10/37 (27%)
<b>ART status*</b>	
Current ART	117/143 (82%)
Months on ART	29 (4-73)
ART regimen: EFV/3TC/TDF	110/117 (94%)
ART regimen: other	7/117 (6%)
Current CPT <sup>†</sup>	98/141 (70%)
<b>Tobacco/alcohol use</b>	
Never tobacco	196/225 (87%)
Ex tobacco	17/225 (8%)
Current tobacco	12/225 (5%)

Table 4.1: Participant Characteristics (*continued*)

Variable	Value
Current alcohol	51/225 (23%)
<b>Education</b>	
Primary incomplete or complete	97/225 (43%)
Secondary school complete	48/225 (21%)
Some secondary education	47/225 (21%)
College or higher	17/225 (8%)
No formal schooling	16/225 (7%)
<b>Employment</b>	
Unemployed	82/225 (36%)
Currently employed	65/225 (29%)
Self-employed	56/225 (25%)
Student	21/225 (9%)
Retired	1/225 (0%)
<b>Toilet facilities</b>	
Pit latrine with slab +/- foot rest	104/225 (46%)
Hanging toilet/latrine	59/225 (26%)
Pit latrine with slab and cover +/- foot rest	45/225 (20%)
Flush Toilet (any type)	14/225 (6%)
No toilet	2/225 (1%)
Composting toilet	1/225 (0%)
<b>Main water source</b>	
Piped outside dwelling	69/225 (31%)
Tube well/borehole	64/225 (28%)
Public tap/standpipe	51/225 (23%)
Piped into dwelling	30/225 (13%)
Unprotected well/spring	5/225 (2%)
Surface water (including rainwater collection)	4/225 (2%)
Tube well with powered pump	2/225 (1%)
<b>Electricity</b>	
Electricity available in house	119/225 (53%)
<b>Main cooking fuel</b>	
Charcoal	161/225 (72%)
Wood	61/225 (27%)
Electricity	3/225 (1%)
<b>Animals at home?</b>	
Any animal	71/225 (32%)
Poultry	46/71 (65%)
Dogs	18/71 (25%)
Goats	12/71 (17%)
Dogs	18/71 (25%)
Other	11/71 (15%)

Table 4.1: Participant Characteristics (*continued*)

Variable	Value
<i>Note:</i>	
ART = Antiretroviral therapy, CPT = Co-trimoxazole preventative therapy, EFV: Efavirenz, 3TC: Lamivudine, TDF: Tenofovir. Numeric values are median (IQR)) unless otherwise stated.	
* ART status includes HIV reactive only as denominator	
† Missing CPT data for two participants.	

Table 4.2: Prehospital healthcare seeking and antimicrobial exposure

Variable	Value
<b>Pre-hospital healthcare seeking</b>	
Sought care prior to attendance at hospital	123/225 (55%)
At health centre	101/123 (82%)
At hospital	16/123 (13%)
At private doctor	8/123 (7%)
Somewhere else	1/123 (1%)
Days prior to today that participant sought care	2 (1-6)
<b>Prehospital antimicrobial exposure</b>	
Received any antimicrobial prior to attendance at hospital	60/225 (27%)
Co-trimoxazole	12/60 (20%)
Ciprofloxacin	10/60 (17%)
Amoxicillin	9/60 (15%)
Ceftriaxone	6/60 (10%)
Metronidazole	5/60 (8%)
Benzylpenicillin	4/60 (7%)
Artesunate	3/60 (5%)
Gentamicin	3/60 (5%)
Erythromycin	2/60 (3%)
LA	2/60 (3%)
SP	2/60 (3%)
Azithromycin	1/60 (2%)
Flucloxacillin	1/60 (2%)
Days prior to today that antimicrobials started	2 (1-5)
<b>Method of transport to hospital</b>	
Minibus	78/225 (35%)
Taxi	65/225 (29%)
Private car/truck	42/225 (19%)
Ambulance	37/225 (16%)
Other	2/225 (1%)
Walk	1/225 (0%)
Cost (MWK) of transport to hospital	1000 (275-3000)

*Note:*

LA = Lumefantrine-artemether, SP = Sulfamethoxazole-pyrimethamine, MWK = Malawian Kwacha. Numeric values are median (IQR)) unless otherwise stated.

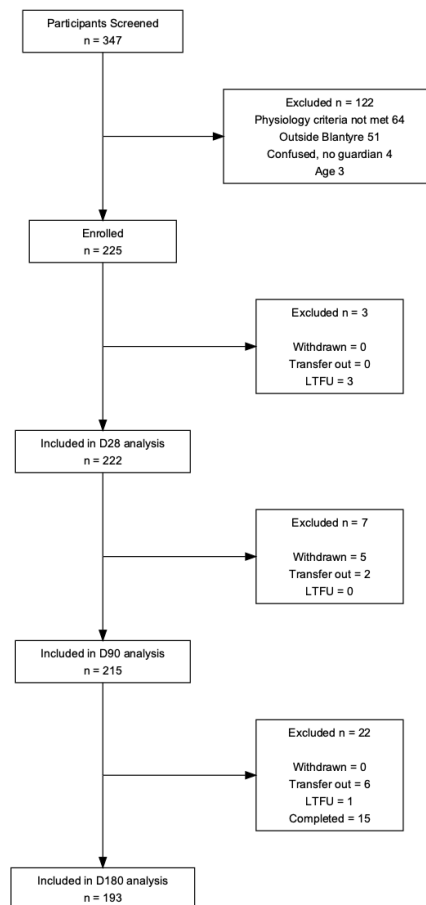


Figure 4.2: Study recruitment and follow up.

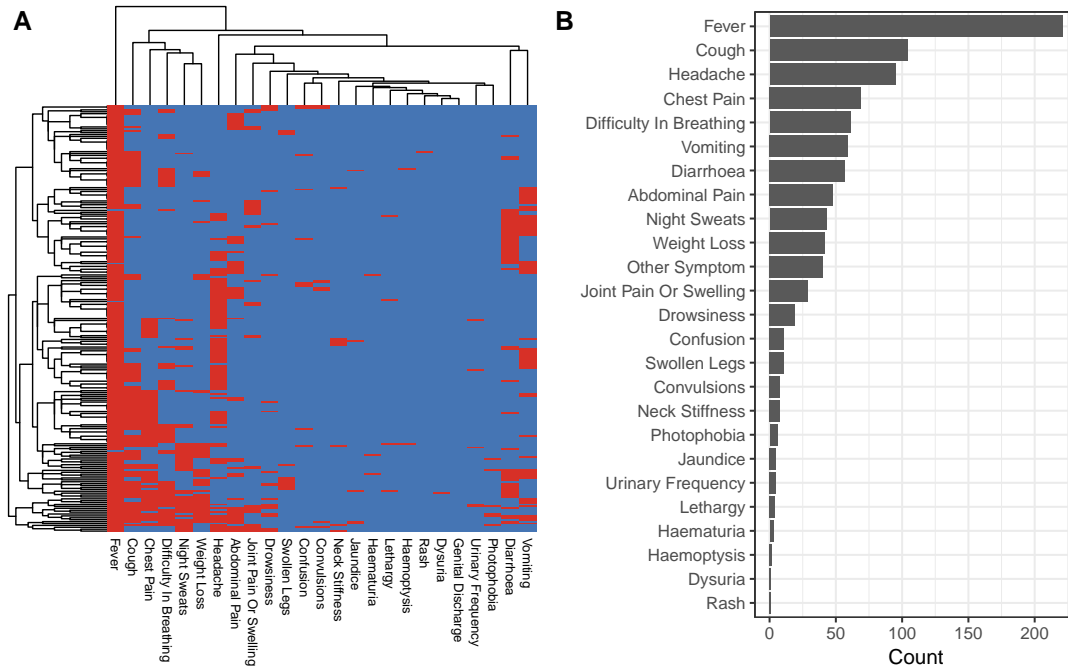


Figure 4.3: Symptoms of recruited participants. A: Row and column clustered heatmap of participant symptoms. Each row represents a patient. Red = presence, blue = absence. B: Frequency of occurrence of symptoms

#### 4.3.3 Admission physiology and laboratory investigations

Admission vital signs and laboratory investigations are shown in Table 4.3. Despite high ART coverage (117/143 [82%]) amongst HIV-infected participants for a median of 29 months, the median (IQR) CD4 count was low at 98 (31-236) cells  $\mu\text{L}^{-1}$ . 108/141 (70%) of participants had a CD4 count below 200 cells  $\mu\text{L}^{-1}$ . CD4 count was similar in participants who had started ART more than 6 months ago as compared to less than three months ago (median [IQR] 99 [27-260] vs 93 [39-137] cells  $\mu\text{L}^{-1}$  respectively) and 42/83 (51%) of participants who had been taking ART for more than 6 months had a CD4 count of less than 100 cells  $\mu\text{L}^{-1}$ , and would fulfil a WHO definition of immunological failure.

Table 4.3: Admission physiology, haematology and biochemistry

Variable	Value
<b>Admission physiology</b>	
Temperature ( $^{\circ}\text{C}$ )	38.5 (37.9-39.0)
Heart rate ( $\text{min}^{-1}$ )	121 (102-132)
Systolic BP (mmHg)	99 (85-119)
Diastolic BP (mmHg)	66 (56-76)
MAP (mmHg)	76 (65-89)
Respiratory rate ( $\text{min}^{-1}$ )	34 (32-38)
Oxygen saturation (%)	96 (94-98)
GCS 15	204/225 (91%)
GCS 11-14	16/225 (7%)
GCS < 11	5/225 (2%)
Unable to stand	63/225 (28%)
<b>Admission CD4 count</b>	
CD4 count* ( $\mu\text{L}^{-1}$ )	98 (31-236)
<b>Admission haematology</b>	
Haemoglobin ( $\times 10^9 \text{ g dL}^{-1}$ )	10.8 (8.2-13.2)
White cell count ( $\times 10^9 \text{ L}^{-1}$ )	6.5 (4.4-11.4)
Neutrophil count ( $\times 10^9 \text{ L}^{-1}$ )	4.0 (2.1-7.5)
Platelet count ( $\times 10^9 \text{ L}^{-1}$ )	218 (146-297)
<b>Admission biochemistry</b>	
Sodium ( $\text{mmol L}^{-1}$ )	134 (130-137)
Potassium ( $\text{mmol L}^{-1}$ )	4.0 (3.6-4.4)
Bicarbonate ( $\text{mmol L}^{-1}$ )	19 (17-22)
Chloride ( $\text{mmol L}^{-1}$ )	101 (97-104)
Urea ( $\text{mmol L}^{-1}$ )	4.8 (3.5-8.0)
Creatinine ( $\text{mmol L}^{-1}$ )	76 (59-103)
Lactate ( $\text{mmol L}^{-1}$ )	3.4 (2.3-5.2)

*Note:*

GCS = Glasgow coma scale, BP = Blood pressure, MAP = Mean arterial blood pressure. Numeric values are median (IQR)) unless otherwise stated.

\* CD4 count includes only HIV-infected participants; 2 values were missing.



#### 4.3.4 Aetiology

In total, 51% (114/225) of the 225 participants had at least one infectious agent identified (Table 4.4), most commonly tuberculosis (76/225 [34%]) followed by bloodstream infection (24/225 [11%]) and malaria (21/225 [9%]). Table 4.5 shows the availability of test and proportion of positive tests across the cohort, stratified by HIV status. 2/225 patients (1%) had a missing aerobic blood culture; the remaining 223 patients had a total of 259 blood cultures performed. 15/259 (6%) blood cultures grew at least one contaminant, but 26 blood cultures from 24 patients were positive for a total of 28 pathogenic bacteria (Figure 4.4): *Salmonella* Typhi was the most commonly isolated pathogenic bacterium, and seemed to show an association with HIV-negative participants: all (8/8) of the participants from whom *S. Typhi* was isolated and whose HIV status was known were HIV noninfected. Of the 18 Gram negative bacteria isolated, 3/18 (17%) were cefpodoxime resistant on AST via disc diffusion testing, and likely ESBL producers: one *K. pneumoniae* and one *E. coli* (both from the same blood culture and same patient) and one *Acinetobacter baumannii*. Both *Staphylococcus aureus* isolates were oxacillin sensitive. The one *Streptococcus pneumoniae* cultured was penicillin intermediate on AST.

Lumbar puncture and CSF culture was carried out in 44 participants: 5/44 (11%) of samples grew a contaminant and no pathogenic bacteria were recovered from any sample. 4/44 (9%) had a detectable cryptococcal antigen (CRAG) in CSF. Malaria testing was missing for 6/225 (3%) of participants, but of the remainder, a positive malaria test was more likely in the HIV-uninfected (12/69 [17%] vs 6/138 [4%],  $p = 0.01$  on pairwise Fisher's exact test). Positive aerobic blood culture showed no statistically significant association with HIV, nor did positive CSF testing, though in the latter case numbers were small and all positive tests (all positive CRAG) were in fact in the HIV-infected (Table 4.5).

Testing for TB, with the exception of sputum Xpert testing, was restricted to HIV-infected participants. Sputum Xpert was carried out in 44/225 (20%) of participants, and was more commonly carried out in the HIV-infected: 35/143 [24%] of HIV-infected participants had sputum testing performed vs 8/70 (11%) of HIV uninfected ( $p = 0.07$  by Fisher's exact test). 53 sputum samples were sent in total from the 44 patients, and 8/44 (18%) diagnoses of TB made, all except one in HIV-infected participants. One sample identified a rifampicin resistance gene; the remainder of infections were rifampicin-sensitive.

155 participants were eligible for urinary lipoarabinomannan (uLAM) and mycobacterial blood culture testing, being either HIV-infected ( $n=143$ ) or of unknown HIV status ( $n=12$ ). Urine was available for 145/155 (94%) of those eligible, and 74/145 (51%) of samples were positive for uLAM. 150/155 (97%) of eligible participants had blood samples collected and cultured

Table 4.4: Final diagnosis of all participants

Diagnosis	Proportion of participants
Tuberculosis	76/225 (34%)
Bloodstream infection	24/225 (11%)
Malaria	21/225 (9%)
Meningitis	4/225 (2%)
No diagnosis	111/225 (49%)

Table 4.5: Diagnostic tests performed and results, stratified by HIV status.

Test	HIV status			All	p
	Positive	Negative	Unknown		
Number of participants	143	70	12	<b>225</b>	-
<b>TB diagnostics</b>					
Urinary LAM	70/136 (51%)	-	4/9 (44%)	<b>74/145 (51%)</b>	-
Sputum Xpert	7/35 (20%)	1/8 (12%)	0/1 (0%)	<b>8/44 (18%)</b>	0.835
TB blood culture	7/128 (5%)	-	1/10 (10%)	<b>8/138 (6%)</b>	-
<b>Other diagnostics</b>					
Aerobic blood culture	13/141 (9%)	9/70 (13%)	2/12 (17%)	<b>24/223 (11%)</b>	0.647
CSF culture or CRAG	4/31 (13%)	0/12 (0%)	0/1 (0%)	<b>4/44 (9%)</b>	0.445
Malaria RDT	6/138 (4%)	12/69 (17%)	3/12 (25%)	<b>21/219 (10%)</b>	0.007

*Note:*

LAM = Lipoarabinomannan, CSF = Cerebrospinal fluid, CRAG = Cryptococcal antigen, RDT = Rapid diagnostic test. p-values are chi-squared test across the three HIV status strata, and hence may be different from the pairwise exact Fisher's tests presented in the text. Urinary LAM and TB blood culture were not carried out in HIV negative participants.

for mycobacteria. 12/150 (8%) grew contaminants and are excluded from the denominators in Table 4.5; of the remainder 8/138 (6%) grew mycobacteria, all *M. tuberculosis*.

Figures 4.6 and 4.5 show the overlap of positive tests from the different diagnostic modalities. Of the 114 patients with at least one positive diagnostic test, 90/114 (79%) had only one positive diagnostic test. The exceptions to this were mycobacterial blood culture and sputum Xpert: patients who had TB diagnosed by these tests tended to also have a positive uLAM. 2/4 (50%) of patients with positive CSF testing (all of whom had detectible CRAG) had also grown *Cryptococcus neoformans* in aerobic blood culture. 111/225 (49%) of patients remained with no diagnosis.

#### 4.3.5 Treatment

At least one antimicrobial drug was received by 95% (214/225) of the cohort during their admission (Table @ref:(time-to-ab-table)), most commonly an antibacterial (207/225 [92%]), but also a significant minority received antitubercular therapy (63/225 [28%]). Of those

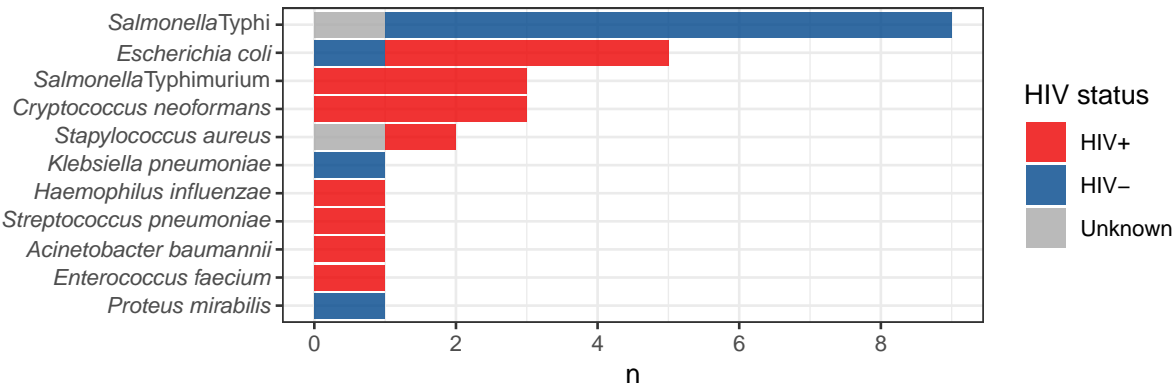


Figure 4.4: Pathogenic isolates recovered from aerobic blood culture. 26 blood cultures in 24 participants were positive for 28 pathogens in total

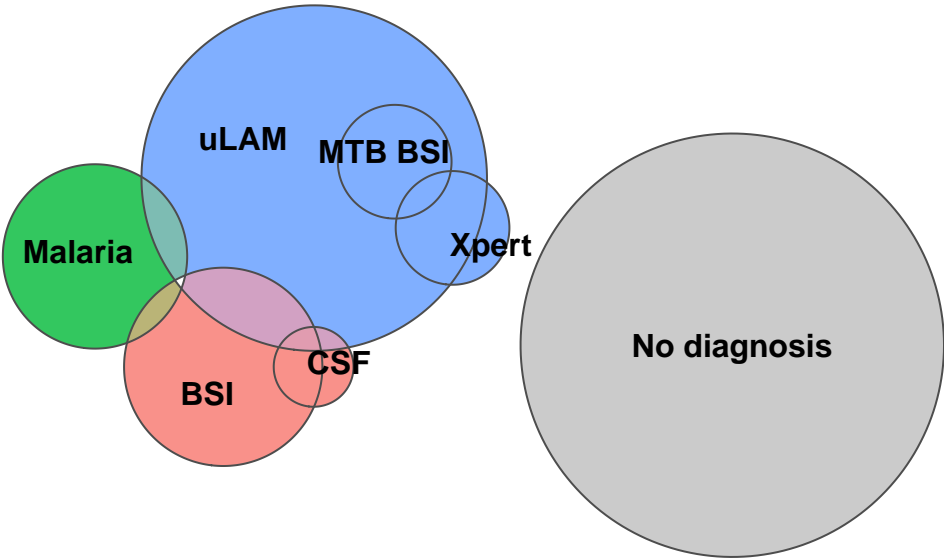


Figure 4.5: Venn diagram showing overlap of positive diagnostic tests; culture of blood and CSF shown in red, malaria in green and TB diagnostics in blue. The CSF variable in includes either a positive culture for a pathogenic bacteria or positive cryptococcal antigen, BSI a positive aerobic culture of pathogenic bacteria from blood and MTB BSI a positive mycobacterial culture of tuberculosis from blood. BSI: Bloodstream infection, CSF: Cerebrospinal fluid, mRDT: Malaria rapid diagnostic test, MTB BSI: Mycobacterium tuberculosis bloodstream infection, uLAM: urinary lipoarabinomannan.

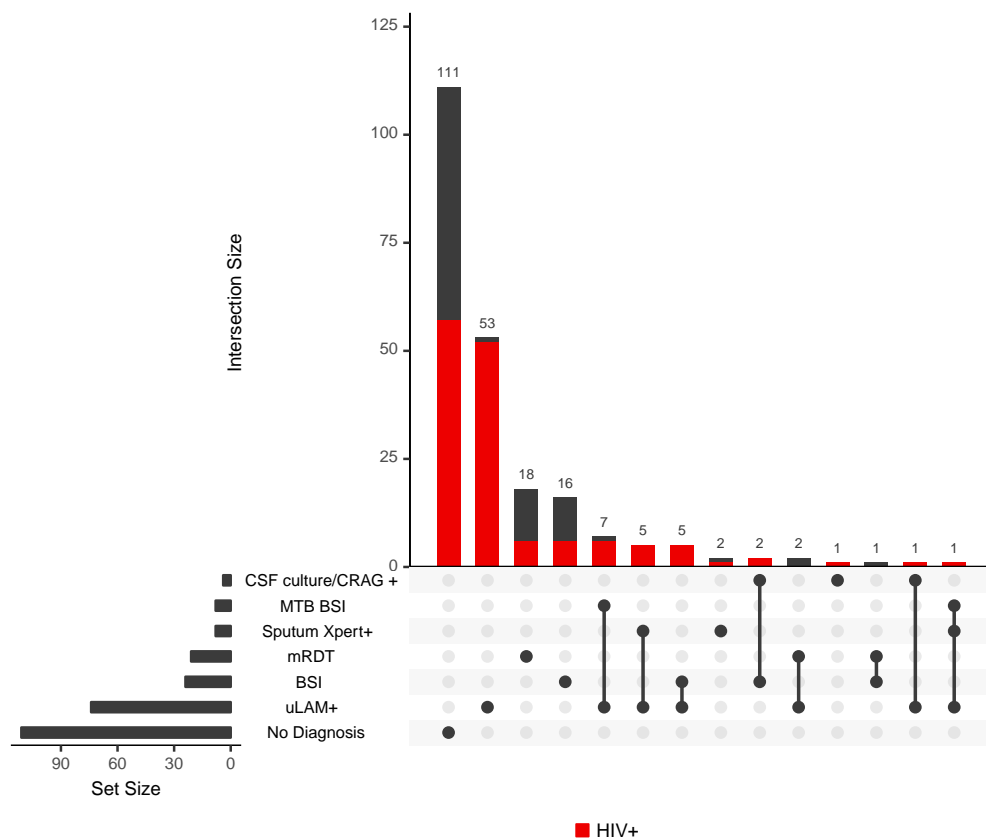


Figure 4.6: UpSet plot of overlap of positive diagnostic tests, showing that for the majority of participants, one test alone is positive. Red colour indicates HIV-infected; black is a composite of HIV-negative and unknown. The CSF variable includes either a positive culture for a pathogenic bacteria or positive cryptococcal antigen, BSI a positive aerobic culture of pathogenic bacteria from blood and MTB BSI a positive mycobacterial culture of tuberculosis from blood. BSI: Bloodstream infection, CSF: Cerebrospinal fluid, CRAG: Cryptococcal antigen, mRDT: Malaria rapid diagnostic test, MTB BSI: Mycobacterium tuberculosis bloodstream infection, uLAM: urinary lipoarabinomannan.

Table 4.6: Door-to-antimicrobial times.

Antimicrobial class	No. participants	Median [IQR] time (hours)
Antibacterial	207/225 (92%)	5.3 (3.7-10.8)
Antitubercular	63/225 (28%)*	120.9 (63.7-171.0)
Antifungal	26/225 (12%)	47.7 (27.9-73.9)
Antimalarial	12/225 (5%)	4.5 (3.1-21.7)

\* 10/63 participants who received antitubercular agents during admission were taking them prior to admission; they are excluded from the calculation of median door-to-antimicrobial time for this class.

receiving antitubercular therapy, 16% (10/63) were taking the medication prior to admission, and the remainder were initiated on therapy during admission. The first antibacterial agent administered was most often ceftriaxone, in 87% (181/207) of cases but ciprofloxacin (18/207 [9%] of participants), amoxicillin (6/207 [3%]) and metronidazole (2/207 [1%]) were also used. Median door to antimicrobial time was 5.3 (IQR 3.7-10.8) hours for antibacterials and 4.5 (IQR 3.1-21.7) hours for antimalarials but longer for antifungals at 47.7 (IQR 27.9-73.9) hours and longer still for antitubercular therapy at 120.9 (IQR 63.7-171.0). Cumulative incidence curves for administration of the different antimicrobial classes are shown in Figure 4.7A-D.

Of all participants, 85% (192/225) received any intravenous fluid in the first 6 hours of enrollment to the study; of these, most received 0.9% saline (160/192 [83%] of those receiving fluid) but 5% dextrose (91/192 [57%]) were also used; Ringer's lactate (6/192 [6%]) and blood (2/192 [1%]) were rarely administered. Of the 192 patients who were administered any fluid, a median of 1.5L (IQR 1.0-2.0L) was administered over the 6hr study period; fluid administration as a function of time is shown in Figure 4.7E.

#### 4.3.6 Outcome

Median hospital stay was 4 (IQR 1-9) days. Mortality of the cohort was 18% (95% CI 13-23%) at 28 days, 24% (95% CI 18-30%) at 90 days and 31% (95% CI 25-38) at 180 days, and higher in HIV-infected participants at each time point (Table 4.7), though not statistically significant on pairwise Fisher's exact test (HIV-infected vs noninfected 19% vs 13%, [p = 0.14] at 28 days, 27% vs 17%, [p = 0.44] at 90 days and 36% vs 21% [p = 0.29] at 180 days). Kaplan-meier estimation of the survival function (Figure 4.8) showed a precipitous decline in survivorship to around day 30 and mortality at a reduced rate thereafter, to the end of the study period. Stratifying the analysis by HIV status revealed that early deaths (within the first 1-2 weeks) occur at similar rates in the two groups before the curves diverge; log-rank test suggested a significant difference in survival function between the two groups (p = 0.03).

Health related quality of life measures, as assessed by EQ-5D-3L, are shown in Figure 4.9 for

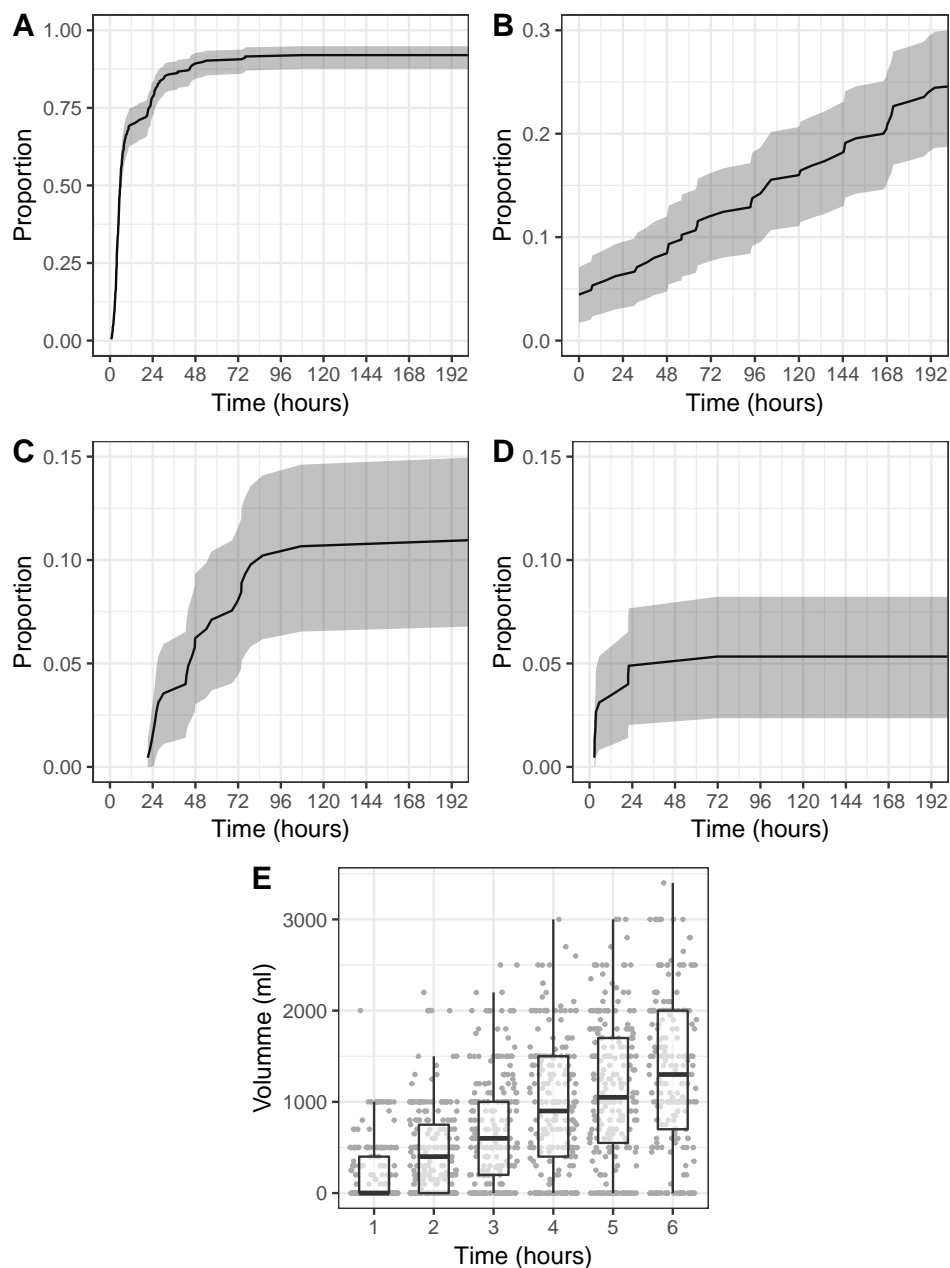


Figure 4.7: Timing of antimicrobial and fluid administration. A-D: Cumulative incidence of administration of antibacterial (A), antitubercular (B), antifungal (C) and antimalarial (D) agents as a function of time since arrival at hospital in hours. E: Total volume of administered intravenous fluid as a function of time since enrollment to study in hours. Boxplots show median, quartiles box and 1.5 times interquartile range as whiskers. Points are jittered around the hour at which they were measured to show distribution.

Table 4.7: Day 28, 90 and 180 mortality stratified by HIV status

	HIV+		HIV-		HIV Unknown		Total	
	n	Mortality	n	Mortality	n	Mortality	n	Mortality
Day 28	143	19% (13-26)	67	13% (6-24)	12	25% (5-57)	<b>222</b>	<b>18% (13-23)</b>
Day 90	139	27% (19-35)	64	17% (9-29)	12	25% (5-57)	<b>215</b>	<b>24% (18-30)</b>
Day 180	125	36% (28-45)	58	21% (11-33)	11	27% (6-61)	<b>194</b>	<b>31% (25-38)</b>

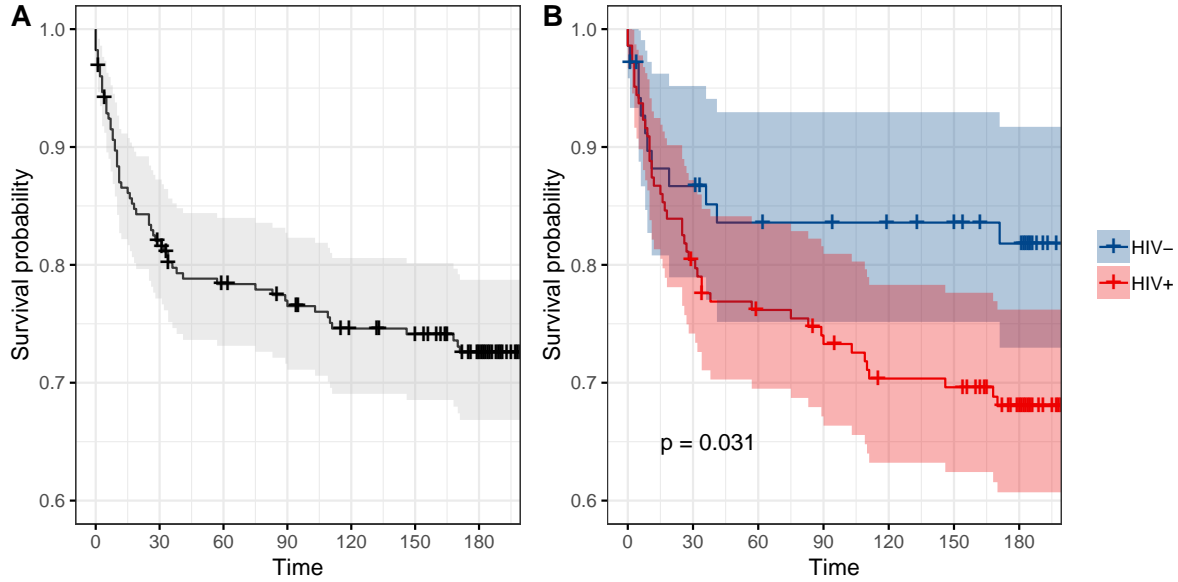


Figure 4.8: Kaplan-Meier survival curves of all included participants (A) and stratified by HIV status (B). Crosses indicate censoring. p value from log-rank test comparing survival of HIV-infected to HIV-noninfected participants shown ( $p = 0.03$ ).

participants with sepsis and the community cohort as a comparator. Acutely, participants with sepsis reported were significantly disabled, reporting at least moderate impairment across all domains in the majority of cases, and over 90% of participants reporting at least moderate impairment in activities of daily living and experiencing at least moderate pain or discomfort. However, recovery following treatment in survivors was rapid. The mean EQ-5Q utility score (a measure of the weight compared to a health state compared to 1, perfect health) of healthy community controls was 0.910 (SD 0.102) at enrollment, significantly higher than participants with sepsis at enrollment (utility score 0.496 (SD 0.251),  $p = < 0.0001$  versus community scores by t-test), but comparable to participants with sepsis at their 12 week assessment (0.913 (SD 0.147),  $p = 0.903$  versus community enrollment scores).

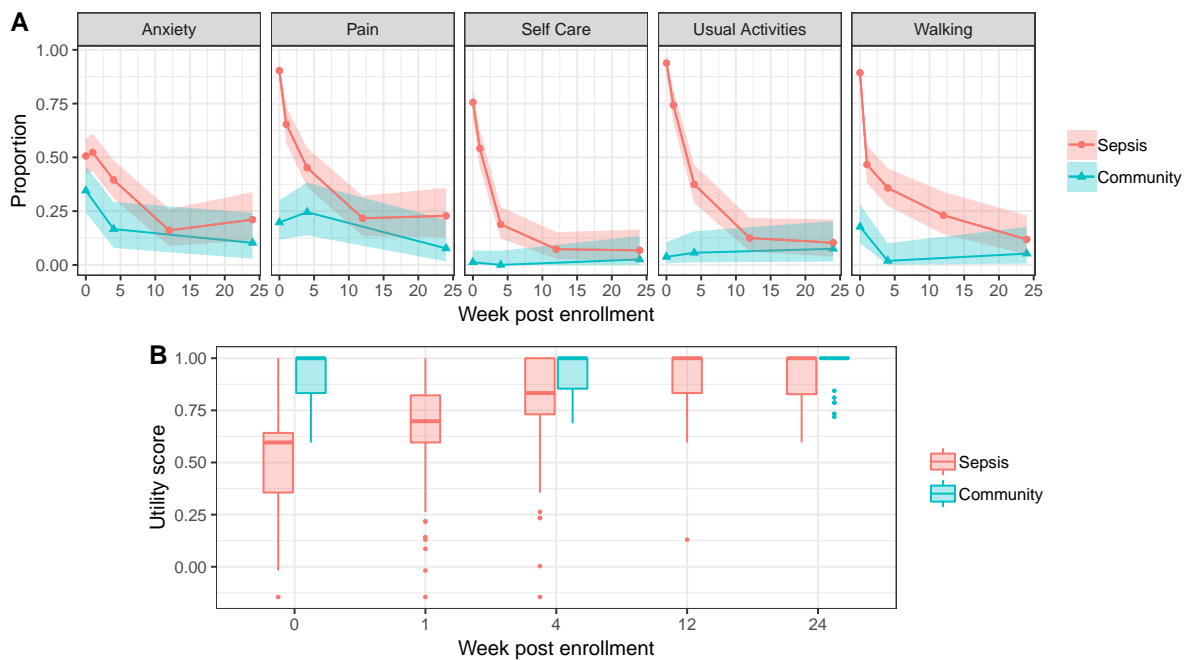


Figure 4.9: Health-related quality of life following sepsis admission, compared to community controls, showing a return to usual quality of life by 12 weeks following admission. A: proportion of participants across each of the five domains of the EQ-5D questionnaire who report at least moderate impairment. B: EQ-5D utility score derived using the Zimbabwean tariff set. The utility score is interpreted as the weight attached to a health state compared to perfect health, which is assigned a value of 1. By 12 weeks there is no statistically significant difference between sepsis and baseline community participant utility scores ( $p = 0.90$  by t-test).



### 4.3.7 Determinants of mortality

Bivariate associations of mortality are shown in 4.8 with variables grouped into putative host, severity, diagnosis and treatment variables. Variables associated with immunosuppression - CD4 count and haemoglobin - were associated with death in bivariate analysis, as were well recognised markers of disease severity: shock, hypoxia, reduced conscious level, hyperlactataemia, and inability to ambulate, as were reduced venous bicarbonate and increased venous urea. A diagnosis of malaria was strongly associated with survival; none of the 21 participants with a diagnosis of malaria died. Conversely, a diagnosis of meningitis was associated with mortality (Table 4.8 and Figure 4.10) though numbers were small ( $n = 4$ ) and so mortality estimates have wide confidence intervals. Receipt of antibacterials, antifungals or antimalarials showed no association with mortality, though almost all participants received antibacterials and only a minority antimalarials and antifungals, so moderate effect sizes would be unlikely to be detected. However, receipt of antitubercular therapy was associated with survival: 8% (4/53) of participants receiving TB therapy died compared to 21% (35/169) who did not receive it ( $p = 0.04$  by Fisher's exact test). This is clearly a finding that is very open to confounding so the associations of receipt of TB treatment were explored (Table @ref:(bivariate-sev-tb-rx) in the appendix to this chapter); patients who received TB therapy were almost all HIV-infected (88% [46/52] vs 60% [95/161] in the no-TB therapy group,  $p < 0.001$ ) with lower CD4 count (median 60 vs 123 cells  $\mu\text{L}^{-1}$ ,  $p = 0.006$ ) and Haemoglobin (median 9.7 vs 11.1 g  $\text{dL}^{-1}$ ), and received more antimalarials (11% [6/53] vs 3% [6/172],  $p = 0.037$ ) and IV fluids (median 1.5L vs 1.2L,  $p = 0.02$ ), though most of these associations would be expected to pull an estimate of the mortality effect of TB therapy towards the null, rather than inflate an effect size. More patients with a positive diagnostic test for TB received TB therapy, as might be expected (53% [28/53] of those receiving TB therapy had a positive diagnostic test for TB, versus 28% [48/172] not receiving therapy,  $p = 0.001$ ), though almost all the TB treatment was empiric, as the treating clinicians did not have access to urinary LAM results (which were batch processed on frozen urines) or mycobacterial blood culture results (which take up to 6 weeks to become positive). To explore the possibility that the apparent association of TB therapy with survival is driven by an effect in those with a diagnosis of TB only, crude mortality estimates stratified by both TB diagnosis and receipt of anti-TB therapy are shown in Figure @ref(fig: mort-strat-tb-and-tb-rx); reduced mortality of TB therapy was in fact apparent (and seemed of similar magnitude) in groups with and without a positive TB diagnostic test. In contrast, mortality benefit of TB therapy did seem to be more pronounced in advanced immunosuppression, defined by a CD4 count of less than 50 cells  $\mu\text{L}^{-1}$  (Figure @ref(fig: mort-strat-tb-and-tb-rx)B)

Kruskal-Wallis test of time to treatment with all antimicrobials found no association with

28-day mortality, and no association with volume of intravenous fluid administered over 6 hours. Further exploration of bivariate associations of mortality with these continuous treatment variables are shown in Figure 4.10, where LOESS moving linear regression provides a nonparametric estimate of probability of death by 28 days as a function of treatment variables. Time to antimalarial therapy is not shown in this plot as no patient who received antimalarial therapy died. No relationship is apparent, thanks to wide confidence intervals except possibly for antibacterial therapy (Figures 4.10D and E): any effect would seem to be only apparent on delay on antimicrobial administration beyond around 40 hours post-admission, with likely non-linearity in effect size as a function of antimicrobial delay. Similarly, volume of intravenous fluid administered does have no apparent effect on 28 day mortality (Figure 4.10B). It might be expected that any effect would be most apparent in participants with shock: stratifying the analysis by shock (defined as mean arterial blood pressure below 75mmHg, Figure (Figures 4.10C) once again showed no apparent relationship.

To explore these associations further, I used a logistic regression analysis, with a primary aim of describing the effect of treatments administered (antimicrobials and fluid) on mortality though as described above, there were several challenges. The problems of collinearity and variable selection were addressed with dimensionality reduction using factor analysis of mixed data (FAMD) on host and severity variables. First, temperature, white cell count, urea, creatinine, lactate were transformed with natural logarithms as their distribution was non-normal on inspection of histograms and kernel density plots. The distributions of oxygen saturation and GCS were very non-normal so were dichotomised into two categories each: GCS as either 15 or less than 15, and oxygen saturation as either above 92% or equal to or below 92%. The composition of first 3 FAMD dimensions in terms of squared correlation ratio (for categorical variables) and the squared correlation coefficient (for continuous variables) are shown in Figure ?? and explained 39% of the variance in the dataset of 18 variables. Furthermore, the dimension provides some discrimination in terms of mortality (Figure ??C) and dimensions one and two provide some discrimination in terms of diagnosis, particularly between TB and malaria (Figure ??D and E).

The first three FAMD dimensions, along with diagnosis and treatment variables were used as explanatory variables in a logistic regression model to predict mortality, though the interest was primarily to correct effect size estimates for confounding rather than predicting outcome. Dimensionality reduction was not undertaken on diagnosis variables as they are largely orthogonal, and also to maintain interpretability. Because no patients with malaria died, the standard maximum likelihood estimation of a logistic regression model failed, so Bayesian logistic regression with weakly informative priors was used following imputation of missing data to form 10 imputed datasets, as described in methods, above. MCMC diagnostics showed good sampling of the posterior:  $\hat{R}$  was less than 1.1, traceplots showed good mixing of chains

(Figure @ref:(model-trplots) in the chapter appendix) and there were no divergences of the sampler. Parameter estimates and 95% credible intervals from this model are shown in 4.9, and conclusions from univariate associations are largely unchanged: we can be confident that malaria is associated with survival, meningitis with death (though with very wide credible intervals reflecting the small number of cases), and administration of TB therapy with survival, following adjustment for the included confounders. The dataset is too small to effectively model interaction terms that would be the equivalent of the bivariate subgroup analyses presented above to test the hypothesis that fluid administration would have a different effect in shock, and that TB therapy would have a different effect in confirmed TB; we will have to be content with the unadjusted analysis.

I then went on to model the effect of antibacterial delay, including only patients who received antibacterials ( $n = 207$ ) using both linear models, and, in view of a possible nonlinear effect, second order polynomial models, both in complete case analysis and following imputation of missing data as before. These data were not included in the full model as it would mean discarding the participants who recieved no antibacterials. The estimates of the coefficients of these models are shown in Table 4.10 and the predicted probability of death by 28 days shown in Figure (Figure 4.12. We have low confidence that estimates of effect size of antimicrobial delay were different from 1 from simple linear models and, though interpreting the coefficients of linear models is difficult, the confidence intervals for the polynomial models are so wide that it is not possible to safely rule in or out a late effect of antimicrobial delay.

### 4.3.8 Discussion

#### 4.3.8.1 Demographics and outcome: significant longer-term mortality

In this chapter, I have presented a clinical and microbiologic description of sepsis in adults in Blantyre, Malawi. Inkeeping with sepsis cohorts elsewhere, the participants are young, and predominantly HIV infected. The proportion of HIV-infected participants (67% of those with known HIV status) is comparable to a study of Sepsis-2 defined sepsis which recruited in QECH in 2008/9 (75%) but lower than sSA sepsis studies with the highest prevalence of HIV-infected participants Uganda in 2006[1] (85%) and 2009[2] (86%) and Zambia in 2012-13[3] (90%). Notably, the proportion of participants receiving HIV therapy (82%) is high compared to other sepsis studies in sSA: higher than the 08/09 Malawian study (44%), Uganda (12-24%) and Zambia (51%), which likely reflects both the success of the Malawian ART programme as well as the impressive increases in ART coverage across the continent. Despite this ostensibly high coverage, it is likely that the sepsis presentation is for a high proportion of the participants in this study a manifestation of ART failure as evidenced by

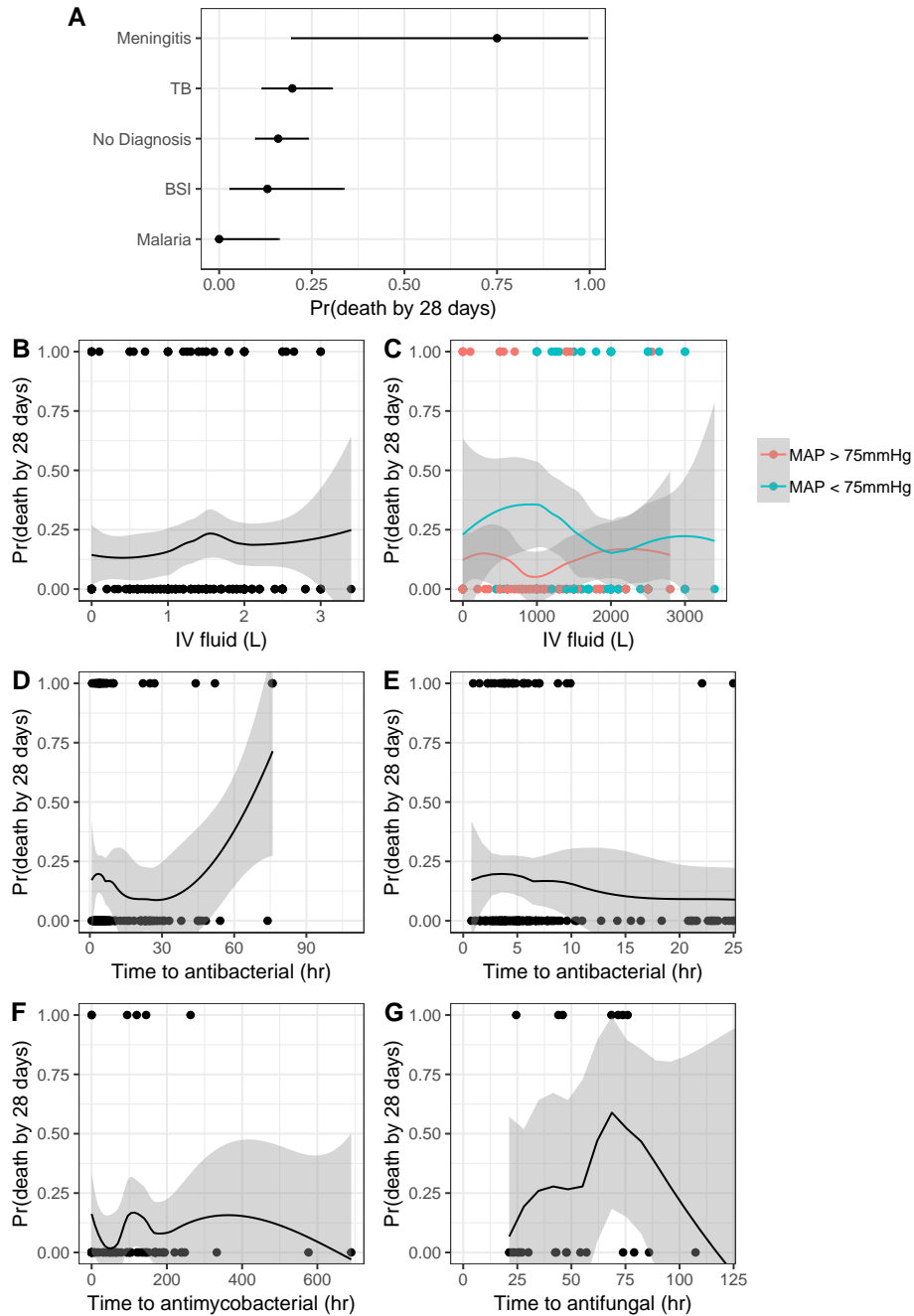


Figure 4.10: Bivariate associations of death by 28 days. A: 28-day mortality stratified by diagnosis. B-G show nonparametric regression (LOESS moving linear regression) of outcome (with death coded as 1 for died and 0 for survived) against various covariates; the regression line can be interpreted as the probability of death by 28 days and can be used to assess for a bivariate relationship and also the nature of any relationship (i.e. linear versus nonlinear. B: IV fluid (L), C: IV fluid stratified by presence or absence of shock (defined as MAP < 75mmHg), D: Time to antibacterials, E: Time to antibacterials with axes restricted to 24hrs to show lack of apparent relationship during first day of admission, F: Time to antimycobacterials, G: Time to antifungals. No plot is shown for antimalarials as no participant receiving antimalarials died. Only D seems to show a relationship, which may be nonlinear.

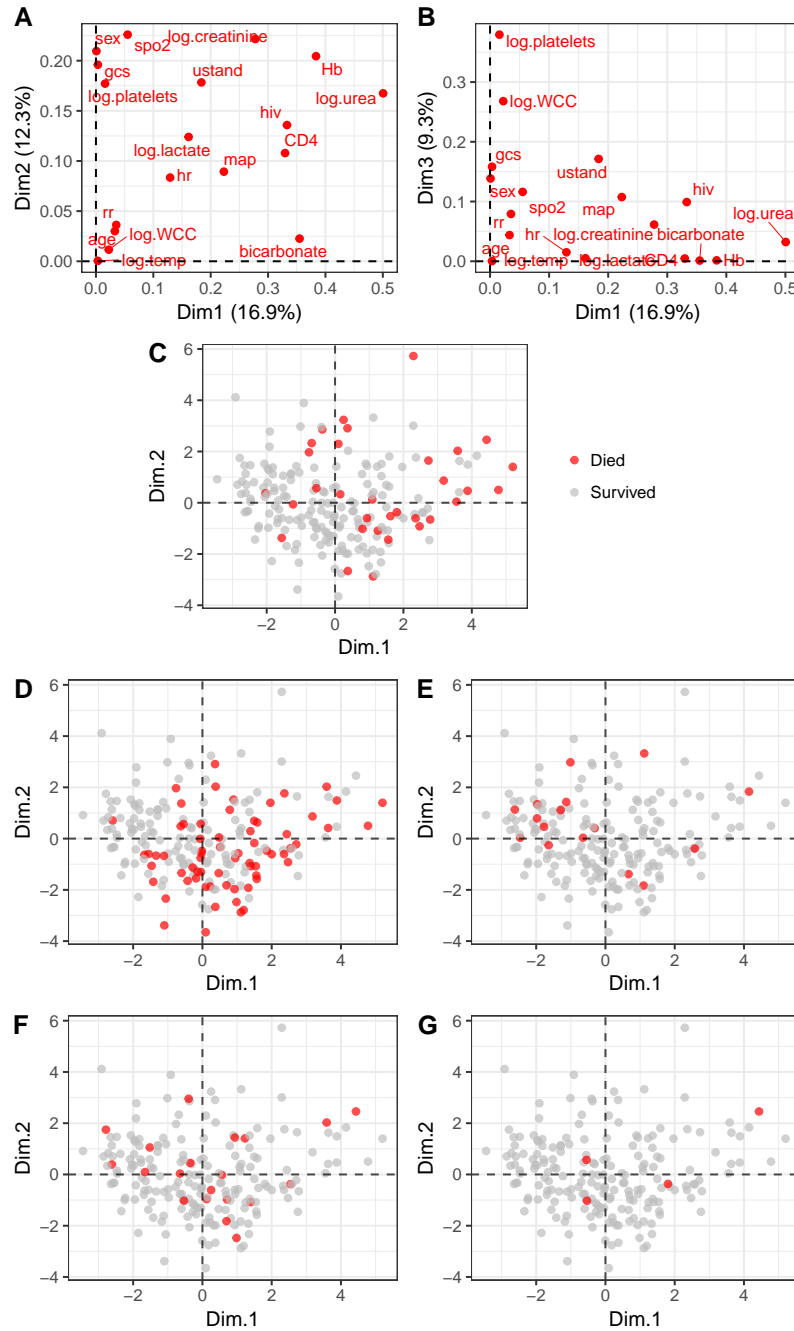


Figure 4.11: Dimensionality reduction of dataset using factor analysis of mixed data (FAMD); this is a combination of principal components analysis (PCA) for continuous variables and multiple correspondence analysis (MCA) for categorical variables, resulting in a new orthogonal coordinate system which maximises explained variance in each FAMD axis. A and B show the squared correlation ratio (for categorical variables) and the squared correlation coefficient (for continuous variables) with dimensions 1 and 2 (A) or 1 and 3 (B), along with the proportion of variance explained by each axis. C shows the location of all individuals in the FAMD space, with patients who died by 28 days coloured red to show that Dim.1 is associated with mortality. D-G show individuals colored by diagnosis: red in each case corresponds to a diagnosis of TB (D), malaria (E), BSI (F) and meningitis (G) to show that malaria and TB separate somewhat in Dim.1 and 2 with malaria patients clustering in top left and TB patients in bottom right

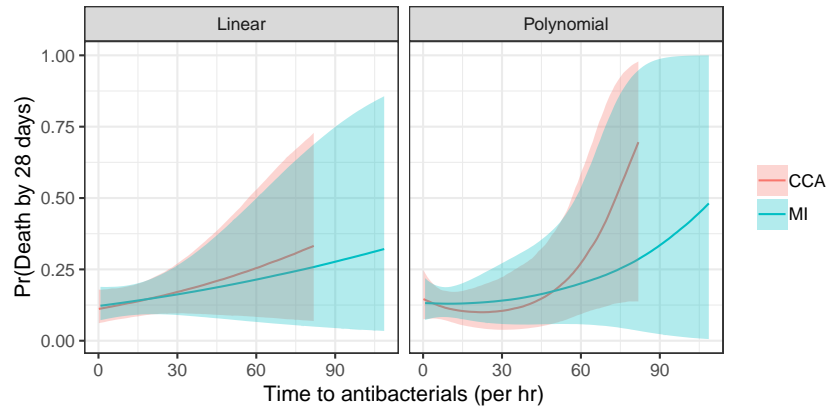


Figure 4.12: Relationship of time-to-antibacterials and 28-day mortality, adjusted for FAMD dimensions 1 and 2. Lines show predicted probability of death from modelling time to antibacterial as a linear predictor of mortality (left panel) and as a second order polynomial (right panel) in both complete case analysis (CCA) and pooled multiply imputed (MI) datasets ( $n=10$ ). In each case there is no clear relationship thanks to uncertainty in the estimate.

the low CD4 cell counts despite ART. Participants had been unwell for some time: a median 7 days. Published data on length of current illness in sepsis is lacking, but what data there are from elsewhere in sSA suggest that this is not unusual[[4];[3];M.A.M.2015; Amir2016]. Barriers to accessing care were not addressed in this study and so the reasons for delaying hospital attendance (including the role of patient and healthcare factors) are not clear; 55% of participants had sought care for their current illness prior to presentation at the hospital, usually at the health centre. Optimum triage and other management of critically unwell patients at the health centre in a resource limited setting is not clear, and is likely to differ from hospital management. This could represent a fruitful area for future research.

The 28-day mortality of the cohort was 18% at 28 days, comparable to the pooled Sepsis-2 sepsis mortality from the systematic review and meta analysis presented in Chapter 1 (23% 95% CI [12-38%]) though considerable lower than the pooled Sepsis-2 severe sepsis mortality (49% 95% CI [39-58]). This is perhaps surprising as the inclusion criteria of this study include organ dysfunction criteria that are more similar to Sepsis-2 severe sepsis definitions than the Sepsis-2 sepsis definitions that are based on the systemic inflammatory response syndrome (SIRS), and would perhaps be expected to result in a higher mortality. In particular, the previous Malawian sepsis study (in the same hospital) from 2008/09 used a SIRS based definition of sepsis and found a mortality of 22%, with a severe sepsis mortality (defined using either 2 SIRS criteria and  $SBP < 90\text{mmHg}$  or any two of  $SBP < 90\text{mmHg}$ , capillary refill time  $> 2\text{s}$ , oxygen saturations  $< 90\%$  or thrombocytopenia) of 50%. There are several possible explanations for this. First, there is likely an effect of differing inclusion criteria: this study includes a respiratory rate criterion for recruitment, which has been shown elsewhere

in sSA in large pooled datasets to be associated with mortality[5], a finding which was not replicated here. Second, sepsis mortality at our centre may be improving, either through improved management, or by population level changes resulting in improved health such as widespread ART coverage or reducing malaria incidence. Certainly, improving sepsis mortality in high-income settings is a trend that has been seen since the pivotal early goal directed therapy trial in 2001[[6];PRISMInvestigators2017;Kaukonen2014]. Third, participants in this study received reasonably intensive monitoring over the first 6 hours of their hospital attendance, which may have contributed to improved processes of care and hence improved outcomes. There is no way to address this hypothesis with the available data.

Participants continued to die post 28-days, to the end of the study period; this was most apparent in HIV-infected participants in whom there was a near-doubling of mortality from 19% at 28 days to 36% at 180 days. To my knowledge, this is the first data on post-30 day outcomes in sepsis in sSA, and demonstrates that longer term mortality following an admission with sepsis is a significant problem. The causes of late (post 28-day) death are unknown from this study. Given the advanced HIV of many of the participants, and the high prevalence of TB, opportunistic infection seems likely, but longitudinal CD4 and viral load measurements were not carried out. Nevertheless, this serves as a reminder of the importance of close clinical and virological monitoring of patients with advanced HIV - an undertaking which is problematic in resource-limited settings - and highlights that an acute sepsis admission can be a presentation of ART failure and an opportunity to intervene to initiate or switch treatment. Given the success of first-line ART roll out in Malawi (and across Africa), this may be an increasingly common scenario.

In contrast to significant medium long term mortality, health-related quality of life (HRQoL, as measured by EQ-5D-3L) seems to return to baseline by 12 weeks following sepsis admission, in contrast to high income settings where longer term morbidity is significant[7]. This may represent differing patient populations with differing levels of physiologic reserve and capacity for recovery from critical illness, as the patient population in this study is significantly younger than a high-income setting sepsis population. It may also reflect the lack of resources available in LMIC: patients who would survive, but with disability, in a high-resource setting may die in a low-resource one. Nevertheless, the rapid return to a comparable HRQoL to healthy community controls following sepsis admission could make improvement of sepsis outcome a cost-effective condition. Once again, to my knowledge, this HRQoL is the first available such data from sepsis in sSA, and can inform health economics analyses in sepsis here: the EQ-5D-3L utility scores can be used to calculate DALYs (disability-adjusted life years) in such an analysis.

#### 4.3.8.2 Aetiology: TB dominates as a cause of sepsis

The aetiology of sepsis in this cohort is dominated by TB, with 34% of participants having at least one positive diagnostic test for TB. The majority of these were positive for urinary LAM. The prevalence of MTB BSI was lower than expected; in previous studies of Sepsis-2 defined severe sepsis in Uganda[1,2] and Zambia[3,4] it was 28-40% in HIV-infected participants. In Malawi in the pre-ART era[8] the prevalence of TB BSI in febrile adults at QECH was 14% (21/173), and 9% (9/104) in 2011 in the same centre in HIV-infected adults admitted with fever and chronic cough[9]. The 6% (8/138) I find here in HIV-infected participants therefore seems low. This could be due to technical (e.g. bottle under or over filling with blood) or laboratory factors, though the latter seems unlikely as the testing was carried out at the same laboratory and with the same SOP as the 2011 study by Feasey et al[9]. This could also be a true finding: given the association of MTB BSI with mortality the lower than expected mortality of this cohort could go hand-in-hand with a lower than expected MTB BSI prevalence, for example, or the high ART prevalence could have an effect on the prevalence of MTB BSI.

Other identified causes of sepsis are as might be expected. *Salmonella* Typhi was the commonest blood stream infection isolate, reflecting the ongoing Typhoid epidemic in Blantyre which began in 2011[10], and seemed to be associated with HIV-uninfected participants, as has been previously described[11]. 51% of the cohort have an unknown diagnosis *Add PHE serology here when back and tidy up - compare unknown diagnosis fraction to eg crump tanzania paper*

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

Using dimensionality reduction and Bayesian logistic regression, I present an assessment of the independent mortality effects of the treatments administered to the cohort, with an aim to inform novel antimicrobial strategies for sepsis in sSA. These approaches were used to deal with the problems of variable selection, collinearity, and nonidentifiability due to separation in logistic regression, and it possible to draw several conclusions from the results. Firstly, there is heterogeneity in outcome across diagnoses: even after controlling for disease severity, malaria was strongly associated with survival to 28 days, and meningitis with death. Mindful of the hypothesised causal structure presented above, this suggests that not all of the mortality risk of death is mediated by the included disease severity markers. The reason for the low mortality of participants with malaria could be due to host factors (partial immunity) or treatment factors (rapid definitive diagnosis using point of care tests) in the context of true malaria disease, or apparent positive tests for malaria could represent incidental parasitaemia.

In high-resource settings, rapid administration of antimicrobials has been shown to be



associated with improved survival in sepsis[12], and all sepsis guidelines stress the importance of rapid administration of antimicrobials[13]. This is based purely on observational evidence and no RCT has ever been (or will be, given the ethical issues) carried out; these studies are all open to confounding and require adjustment for disease severity. In this study, no significant effect of time-to-antibacterials was seen, though it is important not to interpret this lack of detected effect as lack of effect. The largest study to address this question, in a high income setting (New York, USA) found an adjusted odds ratio of 1.04 (95% CI 1.02-1.05) for death per hour delay of antibiotics, and included 49,331 participants[Seymour2017]. Estimates from this study are at least consistent with those, though a lack of precision could be due to an underpowered study. It is also possible, of course, that the beneficial effect of rapid antimicrobials is reduced in a population with a diverse range of causes of sepsis, or a population with a very delayed presentation to hospital, as here. There was some suggestion of a late, possibly nonlinear, deleterious effect of delay of antibacterials after around 40 hours, though confidence intervals were wide and this could represent random variability. Alternatively, antimicrobials started this late after admission could represent hospital-acquired infection, which may well confer a high mortality risk.

There was no detected benefit or harm associated with volume of intravenous fluid administered. How to safely administer intravenous fluid in sSA is unclear after RCTs in children[14] and adults[3] have shown harm to be associated with aggressive fluid resuscitation. Participants in this study received a comparable volume of fluid to the usual care arm of the Zambian RCT in adults[3], given that the trial was recruiting participants with shock: median 2.0L (IQR 1.0-2.5L) by 6 hours versus 1.5L (1.0 - 2.0L) in this study. The intervention arm of the RCT received 3.5L (2.7-4.0L). It may be that participants in this study did not receive enough fluid to be harmful, that there was insufficient variation in fluid exposure to detect an effect on mortality, that the current study is underpowered, or that the Zambian study population differs in some way and so response to fluid is different. Further analysis exploring the effect of rapidity of administration of IV fluid in this cohort is planned.

The most striking finding from the analysis of determinants of mortality is a very strong association between receipt of TB therapy and survival. Care must be taken in interpreting this as cause and effect. Though every attempt has been made to adjust for confounding, in an observational study such as this residual unmeasured confounding is likely. It does seem, however, as though confounding would be likely to bias an estimate of the effect of TB therapy towards the null (in that clinicians might initiate TB therapy on patients who are more unwell and hence more likely to die) rather than producing a spurious protective effect. The benefit of TB therapy was not restricted to those with a confirmed diagnosis of TB, though almost all (88%) of participants who received TB therapy were HIV-infected and care should be exercised in extrapolating to the HIV-uninfected. A protective effect of therapy in sepsis

of patients is plausible from prior studies: autopsy studies show that TB is underdiagnosed in HIV-infected patients who die in hospital[15]. The STAMP trial[16] found a mortality benefit in some a priori subgroups of a strategy of screen-and-treat with urinary LAM for all HIV-infected inpatients, suggesting a significant burden of undiagnosed TB, and prior sepsis cohorts in sSA have found TB as a common cause of sepsis. A retrospective study of 149 HIV infected adults with sepsis in Uganda[17], 55 of whom recieved anti-TB therapy, found an association between receipt of TB therapy and survival in Sepsis-2 severe sepsis (hazard ratio 0.32 95% CI 0.13-0.80 from Cox proportional hazard model) but not Sepsis-2 sepsis (hazard ratio 1.24 95% CI 0.53-2.90), but is hampered by its retrospective design.

What, then, is the role of TB therapy in sepsis in sSA? The fact that the mortality benefit in this study is not restricted to those with a confirmed diagnosis of TB suggests that empiric TB therapy in sepsis or a subset of patients with sepsis (particularly those with a CD4 cell count below 50 cells  $\mu L^{-1}$ , for example) could be beneficial. RCTs of empirical TB treatment have not previously been successful. The REMEMBER trial recruited outpatients with CD4 cell count below 50 cells  $\mu L^{-1}$  and randomised them to isoniazid preventative therapy or full TB therapy, and found no mortality benefit. STASIS found no difference in mortality between a strategy of Xpert and urine LAM screening versus empiric TB therapy in outpatients with CD4 count below 100 cells  $\mu L^{-1}$  and TB Fast Track found no mortality benefit in empiric therapy for outpatients with CD4 cell count below 150  $\mu L^{-1}$  if they were randomised to an algorithm that started TB therapy if they were assessed as high risk for TB using a combination of diagnostic tests (including urinary LAM) and clinical features (including BMI and haemoglobin)[nned ref croi abstract]. However all of these studies recruited ambulatory outpatients; it may be that inpatients have more disseminated TB, or a higher baseline risk of mortality.

The WHO provides guidance on empiric TB therapy. Hospitalised HIV-infected patients in high TB burden settings with cough and so-called “danger signs” (fever  $> 39^{\circ}C$ , inability to stand, respiratory rate above 30  $\text{min}^{-1}$ , heart rate above 120  $\text{min}^{-1}$ ) should receive broad spectrum antimicrobials for 3-5 days, and, if there is no improvement, consider empiric TB therapy. This strategy was developed based largely on expert opinion, has been shown to improve survival compared to usual care in a before-after study in South Africa[18]. Whether a 3-5 day delay will worsen outcomes in critically unwell patients with TB is unknown. There was no apparent relationship seen in this study between time to antitubercular therapy and death, but numbers were small ( $n= 53$ ).

#### 4.3.8.4 Limitations

There are limitations to this study. There is no community control group, so it is not possible to calculate population attributable fractions for pathogens and, in particular, it is not possible to say whether the positive malaria rapid tests in this study represent incidental parasitaemia or disease. Malaria films could perhaps inform this question by quantifying parasitaemia, but were not done. Only one aerobic blood culture and mycobacterial blood culture were done, and both tests are known to have suboptimal sensitivity with only a single culture[19,20]. No anaerobic culture was possible. HIV viral load testing was not done due to resource limitations.

#### 4.3.9 Conclusions and further work

In conclusion, this chapter presents an in-depth clinical and microbiologic assessment of sepsis in Blantyre, Malawi, and finds that the dominant cause is tuberculosis, that long-term mortality is significant, and that empiric TB therapy has a strong protective effect, particularly in advanced immunosuppression. The role of early, empiric TB therapy in sepsis in sub-Saharan Africa is unknown, but these data, I suggest, highlight the potential benefit and provide support for interventional trials.

Further work is planned. Fluid administration in sepsis in sSA is clearly complex, and longitudinal modelling of response to fluid over the first six hours of hospital admission in this cohort is planned. xx% of the cohort still have no diagnosis, and further testing for e.g. Q-fever, Brucellosis, PCP and histoplasmosis could provide insight into the role of these pathogens as causes of sepsis in sSA. The reason for the low prevalence of TB BSI in combination with a high prevalence of urine LAM positivity is unknown, and running Xpert ultra on stored blood samples may help to understand if there were technical problems with the mycobacterial blood cultures.

#### 4.3.10 Appendix

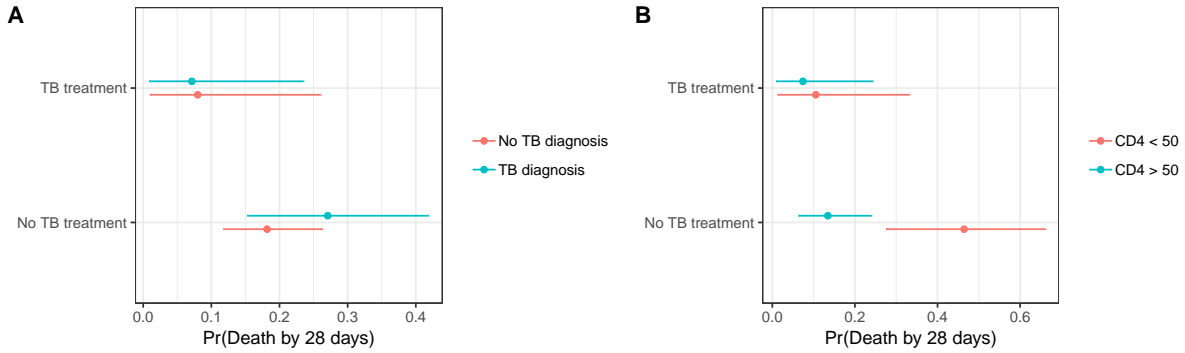


Figure 4.13: Crude 28 day mortality stratified by TB status and receipt of antitubercular therapy: association of TB therapy with survival is not restricted to participants in whom a diagnosis of TB was made.

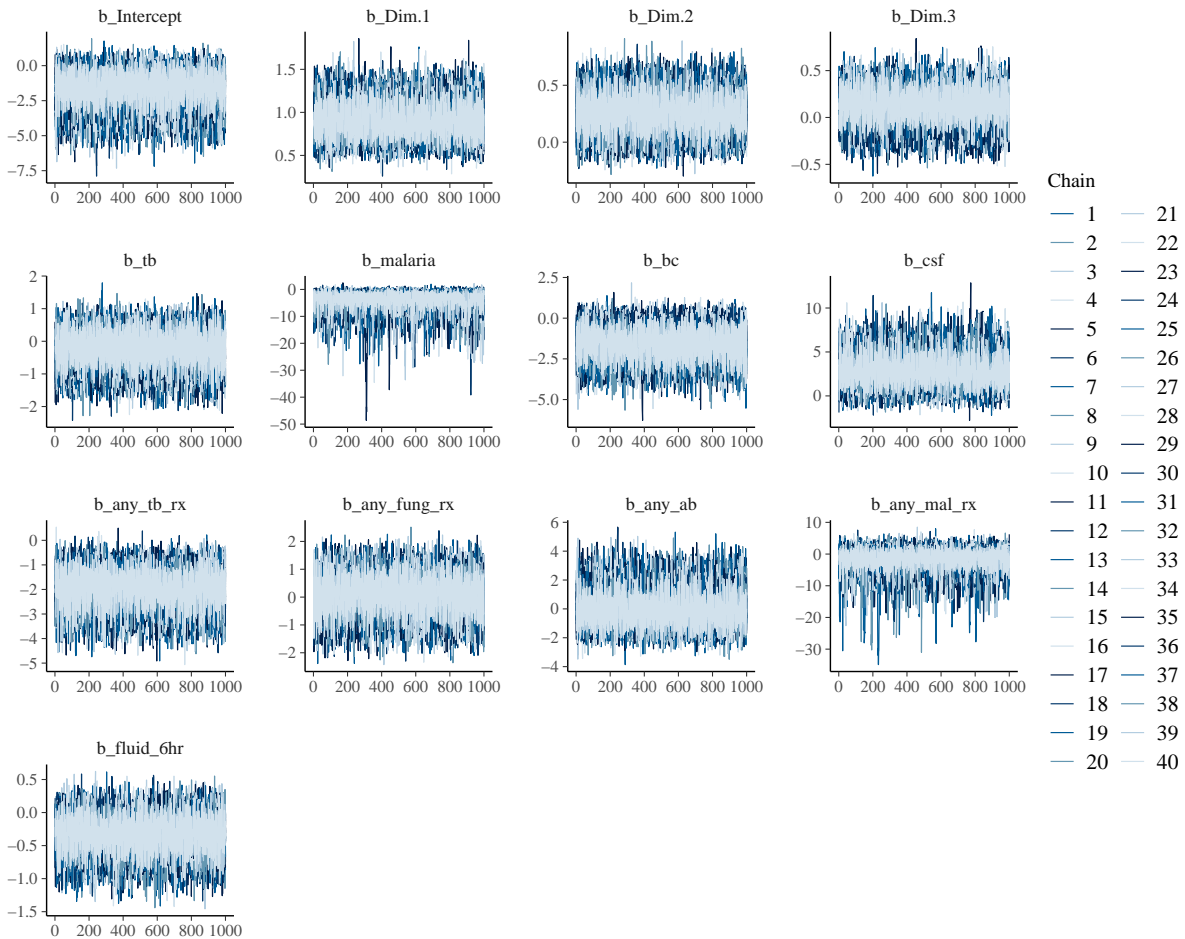


Figure 4.14: Traceplots showing sampling of the posterior for all 10 imputed datasets for the day 28 death outcome model. There are four chains per dataset so 40 chains in total. All show good mixing indicating valid sampling of the posterior. R-hat statistic for all chains was less than 1.1

Table 4.8: Bivariate associations with death by 28 days

Variable	Died	Survived	p
Host Variables			
Age (years)	36.4 (31.5-46.0)	35.9 (27.4-42.9)	0.252
Male sex	19/39 (49%)	93/183 (51%)	0.861
HIV Infected*	27/36 (75%)	116/174 (67%)	0.433
Taking ART†	21/27 (78%)	96/116 (83%)	0.582
<b>CD4 count† (<math>\mu\text{L}^{-1}</math>)</b>	<b>28.5 (9.5-124.5)</b>	<b>103.0 (43.5-251.0)</b>	<b>0.007</b>
<b>Haemoglobin (<math>\times 10^9</math> g dL<sup>-1</sup>)</b>	<b>9.1 (6.0-10.4)</b>	<b>11.0 (8.6-13.4)</b>	<b>&lt;0.001</b>
Severity Variables			
<b>Temperature (<math>^{\circ}\text{C}</math>)</b>	<b>38.1 (37.7-38.8)</b>	<b>38.5 (38.0-39.0)</b>	<b>0.024</b>
Heart rate ( $\text{min}^{-1}$ )	123.0 (104.5-138.5)	120.0 (102.0-131.0)	0.510
<b>Systolic BP (mmHg)</b>	<b>89.0 (76.0-106.0)</b>	<b>99.0 (86.5-118.5)</b>	<b>0.047</b>
<b>Diastolic BP (mmHg)</b>	<b>59.0 (51.0-72.0)</b>	<b>67.0 (57.0-75.5)</b>	<b>0.040</b>
<b>Mean arterial BP (mmHg)</b>	<b>69.7 (60.0-81.3)</b>	<b>78.7 (67.0-89.2)</b>	<b>0.035</b>
Respiratory rate ( $\text{min}^{-1}$ )	34.0 (32.0-36.5)	34.0 (32.0-38.0)	0.720
<b>Oxygen saturation (%)</b>	<b>95.0 (89.5-97.0)</b>	<b>97.0 (95.0-98.0)</b>	<b>0.019</b>
<b>GCS</b>	<b>15.0 (15.0-15.0)</b>	<b>15.0 (15.0-15.0)</b>	<b>0.044</b>
<b>Unable to stand</b>	<b>27/39 (69%)</b>	<b>36/183 (20%)</b>	<b>&lt;0.001</b>
<b>Lactate (mmol L<sup>-1</sup>)</b>	<b>4.6 (3.0-10.6)</b>	<b>3.2 (2.1-4.5)</b>	<b>0.001</b>
White cell count ( $\times 10^9$ L <sup>-1</sup> )	5.9 (3.5-11.0)	6.9 (4.5-11.5)	0.165
Platelet count ( $\times 10^9$ L <sup>-1</sup> )	181.5 (86.8-300.8)	223.0 (148.0-296.5)	0.291
<b>Bicarbonate (mmol L<sup>-1</sup>)</b>	<b>17.0 (14.0-21.0)</b>	<b>20.0 (17.0-22.0)</b>	<b>0.007</b>
<b>Urea (mmol L<sup>-1</sup>)</b>	<b>7.8 (4.5-14.3)</b>	<b>4.5 (3.2-7.0)</b>	<b>&lt;0.001</b>
Creatinine (mmol L <sup>-1</sup> )	90.0 (60.0-185.0)	73.0 (59.0-96.0)	0.100
Diagnosis			
BSI	3/39 (8%)	20/183 (11%)	0.773
TB	15/39 (38%)	61/183 (33%)	0.579
<b>Malaria</b>	<b>0/39 (0%)</b>	<b>21/183 (11%)</b>	<b>0.030</b>
<b>Meningitis</b>	<b>3/39 (8%)</b>	<b>1/183 (1%)</b>	<b>0.018</b>
No diagnosis	21/39 (54%)	88/183 (48%)	0.598
Treatment Received			
Antibacterials	37/39 (95%)	167/183 (91%)	0.746
Time to Antibacterials (hr)	4.7 (3.8-8.8)	5.3 (3.6-10.8)	0.648
Antifungals	7/39 (18%)	19/183 (10%)	0.180
Time to Antifungals (hr)	68.5 (45.0-72.7)	47.6 (26.6-76.4)	0.665
Antimalarials	0/39 (0%)	12/183 (7%)	0.132
Time to Antimalarials (hr)	NA (NA-NA)	4.5 (3.1-21.7)	NA
<b>Antimycobacterials</b>	<b>4/39 (10%)</b>	<b>49/183 (27%)</b>	<b>0.037</b>
Time to Antimycobacterials (hr)	107.3 (23.6-138.7)	99.0 (37.0-169.4)	0.778
IV fluid (L)	1.4 (1.0-2.0)	1.3 (0.6-2.0)	0.368

Note:

BP = Blood pressure, GCS = Glasgow coma scale. Numeric variables are presented as median (IQR) and categorical variables as proportions. P-values are from Kruskal-Wallis test for continuous variables and Fisher's exact test for categorical variables.

\* Participants with HIV status unknown not included in this row

† Includes only HIV-infected participants

Table 4.9: Adjusted odds ratios (aOR) for death by 28 days following multiple imputation of missing data

Variable	aOR
FAMD composite variables	
FAMD Dimension 1	2.53 (1.82-3.70)
FAMD Dimension 2	1.30 (0.97-1.76)
FAMD Dimension 3	1.09 (0.78-1.53)
Diagnosis	
TB	0.72 (0.27-1.83)
Malaria	0.03 (0.00-0.76)
BSI	0.25 (0.04-1.15)
Meningitis	16.18 (1.10-636.25)
Therapies recieved	
Anti-TB	0.14 (0.03-0.47)
Antifungals	1.06 (0.30-3.46)
Antibacterials	1.28 (0.18-14.30)
Antimalarials	0.32 (0.00-16.67)
IV Fluid Recieved (per L)	0.65 (0.35-1.17)

*Note:*

FAMD = Factor Analysis of Mixed Data, BSI = Blood-stream infection, TB = Tuberculosis.

Table 4.10: Coefficient values (expressed as odds ratios) for models predicting death by 28 days that are linear and polynomial in time to antibacterials

Coefficient	Odds Ratio	
	Linear Model	Polynomial Model
FAMD Dimension 1	1.84 (1.45-2.38)	1.84 (1.44-2.38)
FAMD Dimension 2	1.20 (0.92-1.57)	1.19 (0.91-1.55)
Time to antibacterials (hrs)	1.01 (0.99-1.04)	1.00 (0.95-1.05)
(Time to antibacterials) <sup>2</sup>	-	1.00 (0.99-1.02)

*Note:*

FAMD = Factor Analysis of Mixed Data.

Table 4.11: Bivariate associations with receipt of TB treatment

Variable	TB treatment	No TB treatment	p
Host Variables			
Age (years)	37.7 (32.5-42.9)	35.6 (26.8-43.6)	0.487
Male sex	30/53 (57%)	84/172 (49%)	0.349
<b>HIV Infected*</b>	<b>46/52 (88%)</b>	<b>97/161 (60%)</b>	<b>&lt;0.001</b>
Taking ART <sup>†</sup>	35/46 (76%)	82/97 (85%)	0.250
<b>CD4 count<sup>†</sup> (<math>\mu\text{L}^{-1}</math>)</b>	<b>60.0 (26.2-114.8)</b>	<b>123.0 (39.0-274.0)</b>	<b>0.006</b>
<b>Haemoglobin (<math>\times 10^9 \text{ g dL}^{-1}</math>)</b>	<b>9.7 (7.4-11.3)</b>	<b>11.1 (8.6-13.9)</b>	<b>0.001</b>
Severity Variables			
Temperature ( $^{\circ}\text{C}$ )	38.5 (38.0-39.2)	38.4 (37.9-39.0)	0.487
Heart rate ( $\text{min}^{-1}$ )	125.0 (110.0-134.0)	119.5 (99.8-132.0)	0.051
Systolic BP (mmHg)	92.0 (81.0-107.0)	99.0 (86.0-120.0)	0.133
Diastolic BP (mmHg)	67.0 (56.0-71.0)	65.0 (57.0-78.8)	0.486
Mean arterial BP (mmHg)	76.0 (65.3-83.7)	77.2 (65.1-91.2)	0.272
Respiratory rate ( $\text{min}^{-1}$ )	34.0 (30.0-38.0)	34.0 (32.0-37.0)	0.503
Oxygen saturation (%)	96.0 (94.0-98.0)	96.0 (95.0-98.0)	0.871
GCS	15.0 (15.0-15.0)	15.0 (15.0-15.0)	0.566
Unable to stand	13/53 (25%)	50/172 (29%)	0.601
Lactate ( $\text{mmol L}^{-1}$ )	3.2 (2.4-4.9)	3.4 (2.2-5.2)	0.790
White cell count ( $\times 10^9 \text{ L}^{-1}$ )	6.4 (4.6-9.1)	6.6 (4.3-11.7)	0.595
Platelet count ( $\times 10^9 \text{ L}^{-1}$ )	225.5 (146.8-303.2)	215.0 (145.0-296.0)	0.498
Bicarbonate ( $\text{mmol L}^{-1}$ )	18.0 (16.0-21.0)	20.0 (17.0-22.5)	0.065
Urea ( $\text{mmol L}^{-1}$ )	5.0 (3.8-8.7)	4.6 (3.3-7.7)	0.174
Creatinine ( $\text{mmol L}^{-1}$ )	76.0 (59.0-105.0)	75.5 (59.0-102.2)	0.824
Diagnosis			
BSI	4/53 (8%)	20/172 (12%)	0.611
<b>TB</b>	<b>28/53 (53%)</b>	<b>48/172 (28%)</b>	<b>0.001</b>
Malaria	6/53 (11%)	15/172 (9%)	0.592
Meningitis	1/53 (2%)	3/172 (2%)	1.000
<b>No diagnosis</b>	<b>15/53 (28%)</b>	<b>96/172 (56%)</b>	<b>0.001</b>
Treatment Received			
Antibacterials	47/53 (89%)	160/172 (93%)	0.383
Time to Antibacterials (hr)	5.1 (3.8-9.7)	5.4 (3.6-13.4)	0.844
Antifungals	8/53 (15%)	18/172 (10%)	0.337
Time to Antifungals (hr)	45.4 (25.2-60.6)	50.9 (33.2-78.3)	0.243
<b>Antimalarials</b>	<b>6/53 (11%)</b>	<b>6/172 (3%)</b>	<b>0.037</b>
Time to Antimalarials (hr)	4.5 (3.0-11.7)	12.5 (3.3-21.7)	0.631
<b>IV fluid (ml)</b>	<b>1.5 (1.0-2.0)</b>	<b>1.2 (0.5-2.0)</b>	<b>0.020</b>

Note:

BP = Blood pressure, GCS = Glasgow coma scale. Numeric variables are presented as median (IQR) and categorical variables as proportions. P-values are from Kruskal-Wallis test for continuous variables and Fisher's exact test for categorical variables.

\* Participants with HIV status unknown not included in this row

<sup>†</sup> Includes only HIV-infected participants





## Chapter 5

# Early response to resuscitation in sepsis



## Chapter 6

# Gut mucosal carriage of ESBL-E in Blantyre, Malawi



## Chapter 7

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

Placeholder



## 7.1 Chapter overview

## 7.2 Methods

### 7.2.1 Bioinformatic pipeline

### 7.2.2 Global *E. coli* collection

### 7.2.3 Statistical analysis

## 7.3 Results

### 7.3.1 Samples and quality control

### 7.3.2 Phylogroup, MLST and core genome phylogeny of study isolates

### 7.3.3 Study isolates in a global context

### 7.3.4 Antimicrobial resistance determinants

#### 7.3.4.1 $\beta$ -lactam resistance

#### 7.3.4.2 Quinolone resistance

#### 7.3.4.3 Aminoglycoside resistance

#### 7.3.4.4 Chloramphenicol, co-trimoxazole, tetracycline and other resistance determinants

#### 7.3.4.5 Clustering and lineage association of AMR determinants

### 7.3.5 Plasmid replicons

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

#### 7.3.6.1 Hierarchical BAPS clustering of core gene pseudosequences

#### 7.3.6.2 ESBL-clusters

#### 7.3.6.3 Assessing for healthcare-associated lineages

#### 7.3.6.4 Assessing for within-patient conservation of lineage or MGE

## 7.4 Discussion





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