

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

Preface	9
1 Introduction	11
1.1 Chapter Overview	13
1.2 Sepsis in sub-Saharan Africa	13
1.3 ESBL-E in sub-Saharan Africa	13
1.4 Conclusions	13
1.5 Thesis overview	13
1.6 Appendix	13
1.7 References	13
2 Methods	15
2.1 Chapter Overview	17
2.2 Study site	17
2.3 Clinical Study	17
2.4 Diagnostic Laboratory Procedures	17
2.5 Molecular methods	17
2.6 Bioinformatics	17
2.7 Statistical Analysis	17
2.8 Study Team	17
2.9 Data Collection and Storage	17
2.10 Ethical Approval, Consent and Participant Remuneration	17
3 <i>Mycobacterium tuberculosis</i> BSI: an IPD meta analysis	19
4 Sepsis in Blantyre, Malawi	21
4.1 Chapter overview	21
4.2 Methods	21
4.3 Results	21

5	Early response to resuscitation in sepsis	35
6	Gut mucosal carriage of ESBL-E in Blantyre, Malawi	37
7	Whole genome sequencing of ESBL <i>E. coli</i> carriage isolates	39
7.1	Chapter overview	41
7.2	Methods	41
7.3	Results	41
7.4	Discussion	41
7.5	Appendix	41
	References	43

List of Tables

4.1	Participant Characteristics	23
4.2	Prehospital healthcare seeking and antimicrobial exposure	26
4.3	Admission physiology, haematology and biochemistry	28
4.4	Final diagnosis of all participants	30
4.5	Positive diagnostic tests for all participants, stratified by HIV status.	30

List of Figures

4.1	Study recruitment and follow up.	22
4.2	Symptoms of recruited participants	27
4.3	Pathogenic isolates recovered from aerobic blood culture.	31
4.4	Symptoms of recruited participants	31
4.5	UpSet plot of diagnostic testing	32

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-Saharan 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 identification 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

2.4.2.4 Sputum Xpert

Chapter 3

Mycobacterium tuberculosis BSI: an IPD meta analysis

Chapter 4

Sepsis in Blantyre, Malawi

4.1 Chapter overview

4.2 Methods

blah blah

4.3 Results

4.3.1 Study population

Figure 4.1 shows flow through the study. 225 patients were recruited in 20 months between 19th February 2017 and 2nd October 2018. In total, 4 patients (2%) were lost to follow up over the 180-day study period; 5 patients (2%) withdrew; and 7 patients (3%) transferred out of the study area before 180 days. Four of the five patients 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%) patients had their final study visit before 180 days, and so were not included in the 180-day outcome analysis.

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

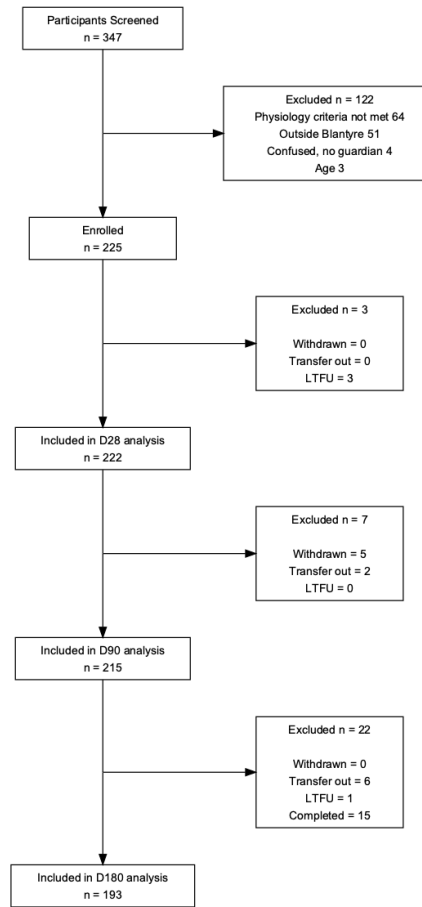


Figure 4.1: Study recruitment and follow up.

regimen of efavirenz, lamivudine and tenofovir, and 88/117 (75%) had been taking ART for more than three months. Figure 4.2 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
Demographics	
Age (years)	36 (28-44)
Male sex	114/225 (51%)
HIV/TB status	
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%)
ART status*	
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 [†]	98/141 (70%)
Tobacco/alcohol use	
Never tobacco	196/225 (87%)
Ex tobacco	17/225 (8%)
Current tobacco	12/225 (5%)
Current alcohol	51/225 (23%)
Education	
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%)
Employment	
Unemployed	82/225 (36%)
Currently employed	65/225 (29%)
Self-employed	56/225 (25%)
Student	21/225 (9%)
Retired	1/225 (0%)
Toilet facilities	
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%)
Main water source	
Piped outside dwelling	69/225 (31%)

Table 4.1: Participant Characteristics (*continued*)

Variable	Value
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%)
Electricity	
Electricity available in house	119/225 (53%)
Main cooking fuel	
Charcoal	161/225 (72%)
Wood	61/225 (27%)
Electricity	3/225 (1%)
Animals at home?	
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%)

Note:

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.

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 μL^{-1} . 108/141 (70%) of participants had a CD4 count below 200 cells μL^{-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 μL^{-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 μL^{-1} , and would fulfil a WHO definition of immunological failure.

Table 4.2: Prehospital healthcare seeking and antimicrobial exposure

Variable	Value
Pre-hospital healthcare seeking	
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)
Prehospital antimicrobial exposure	
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)
Method of transport to hospital	
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.

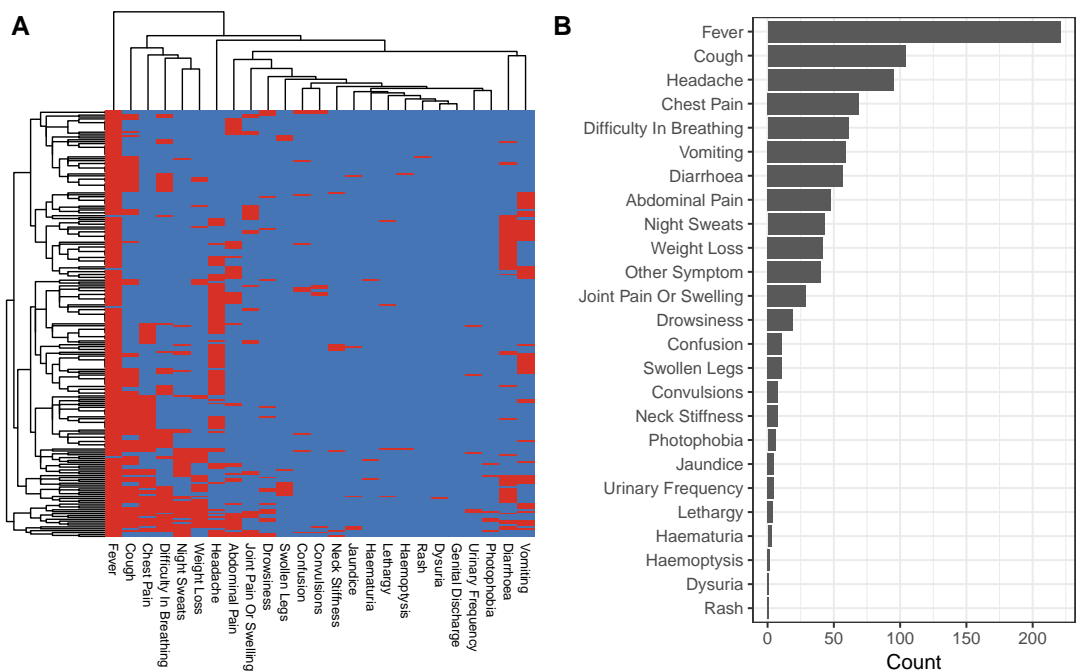


Figure 4.2: Symptoms of recruited participants. A: Row and column clustered heatmap of participant symptoms. Each row represents a patient. Red = presence, blue = absence. B: Frequency of occurrence of symptoms

Table 4.3: Admission physiology, haematology and biochemistry

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

Note:

GCS = Glasgow coma scale. 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 4.3): *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: Positive diagnostic tests for all participants, stratified by HIV status.

Test	HIV status			All	p
	Positive	Negative	Unknown		
Number of participants	143	70	12	225	-
TB diagnostics					
Urinary LAM	70/136 (51%)	-	4/9 (44%)	74/145 (51%)	-
Sputum Xpert	7/35 (20%)	1/8 (12%)	0/1 (0%)	8/44 (18%)	0.835
TB blood culture	7/128 (5%)	-	1/10 (10%)	8/138 (6%)	-
Other diagnostics					
Aerobic blood culture	13/141 (9%)	9/70 (13%)	2/12 (17%)	24/223 (11%)	0.647
CSF culture or CRAG	4/31 (13%)	0/12 (0%)	0/1 (0%)	4/44 (9%)	0.445
Malaria RDT	6/138 (4%)	12/69 (17%)	3/12 (25%)	21/219 (10%)	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 4.5 and 4.4 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

Table: Time to antimicrobials Time to fluid Amount of fluid

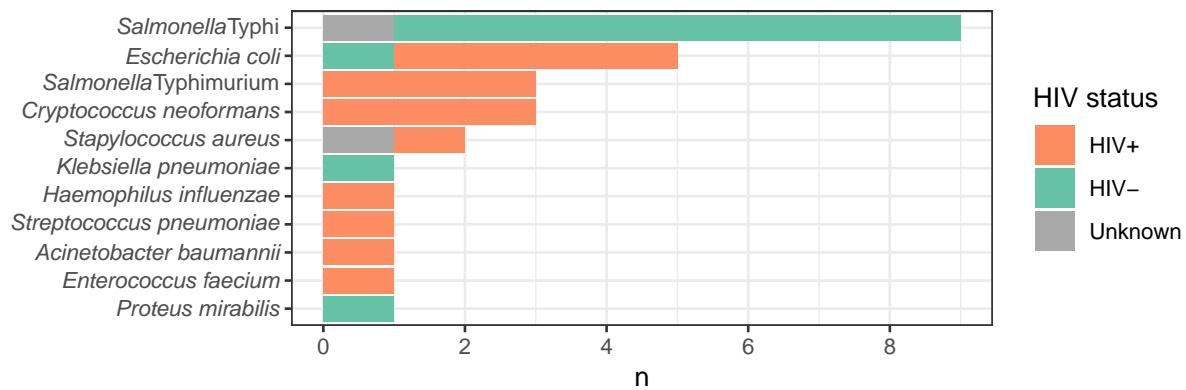


Figure 4.3: Pathogenic isolates recovered from aerobic blood culture. 26 blood cultures in 24 participants were positive for 28 pathogens in total

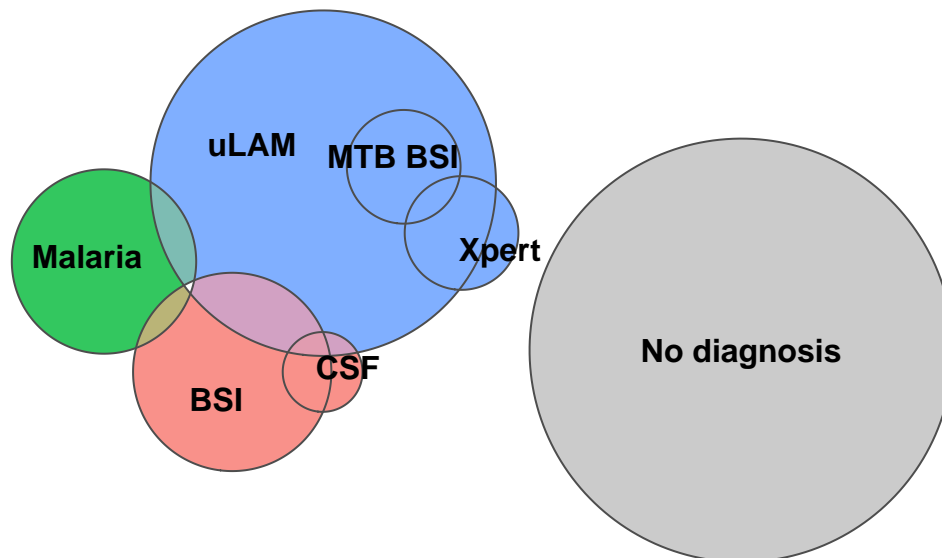


Figure 4.4: Venn diagram showing overlap of diagnostic tests; culture of blood and CSF shown in red, malaria in green and TB diagnostics in blue. The CSF variable includes either a positive culture for a pathogenic bacteria or positive cryptococcal antigen, BSI a positive aerobic culture of pathogenic 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.

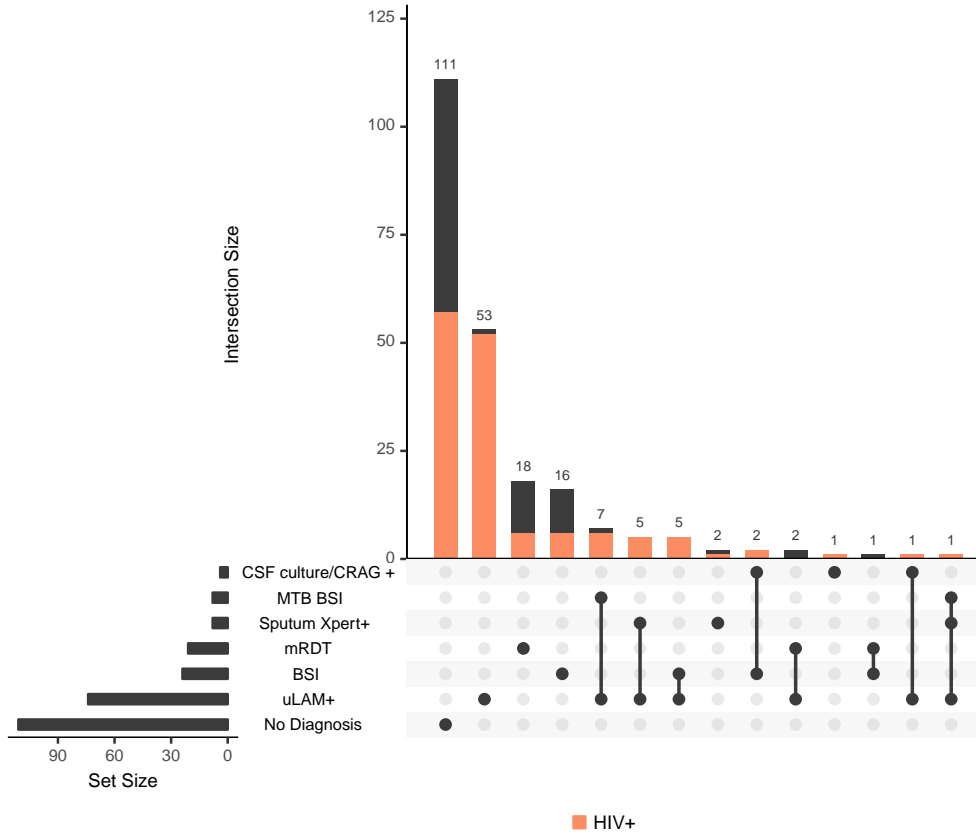


Figure 4.5: UpSet plot of overlap of positive diagnostic tests, showing that for the majority of participants, one test alone is positive. Red colour indicates HIV-infected; black is a composite of HIV-negative and unknown. The CSF variable includes either a positive culture for a pathogenic bacteria 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.

4.3.6 Outcome

Table - 28 and 90 day mortality

Figure - KM survival curve

Logistic regression - determinants of 28 day mortality

Morbidity -

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 β -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

References