Causes and consequences of adult sepsis in Blantyre, 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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Introduction

1.1 Introduction

The syndrome of sepsis has been described since antiquity; from Hippocrates to Galen and Semmelweis, the potentially serious systemic consequences of a localised infection have long been recognized. The word sepsis arises from the Greek $\sigma\eta\psi\iota\zeta$ meaning decomposition and was described by Hippocrates as a dangerous putrefaction in the body[1]. Modern definitions of sepsis conceptualise it as a syndrome of life threatening organ dysfunction due to a dysregulated host response to infection[2], but despite increased understanding of its pathogenesis[3], mortality from sepsis remains high. Progress has been made in improving sepsis mortality in high income settings[4,5], through timely application of basic care[[6]; Seymour2017]: early appropriate antimicrobials, aggressive fluid resuscitation and organ support largely in a critical care environment. Limited data from low resource settings including sub-Saharan Africa (sSA) suggest that mortality remains high[7], and increasing evidence suggests that exporting high-income setting sepsis protocols to sSA has the potential to do harm[8]. Data to guide sepsis management protocols for sSA are urgently needed.

Data on sepsis aetiology from sSA to guide antimicrobial strategies are lacking; currently, in Blantyre Malawi, for example, empirical management of sepsis is the norm and patients often receive prolonged empiric courses of broad spectrum antimicrobials – largely ceftriaxone, a third-generation cephalosporin antibiotic. Limited data suggest that tuberculosis, arboviruses and bacterial zoonoses may be important causes of severe febrile illness in sSA[[9]; [10]; Rubach2015; Crump2013], pathogens which largely go untreated by ceftriaxone. On a population level invasive *Escherichia coli* and *Klebsiella pneumoniae* bacteria are showing an alarming increase in ceftriaxone resistance since the drug was introduced in Malawi in 2005[11,12]. The majority of these resistant bacteria are so-called extended-spectrum

beta lactamase producers (ESBL-producers) and are often untreatable with locally available antimicrobials. Novel antimicrobial strategies are needed to safely preserve ceftriaxone - often a first and last line antibiotic in Malawi - for those who need it.

It is the hypothesis of this thesis, then, that sepsis in Malawi is caused by a wide variety of infections that are currently unrecognised and untreated, and that this is contributing to high sepsis mortality. Conversely, prolonged ceftriaxone exposure in sepsis survivors is causing acquisition and carriage of resistant bacteria (principally ESBL Enterobacteriaceae, henceforth ESBL-E) and their transportation into the community. I ask the question as to whether novel antimicrobial strategies in sSA can not only improve outcomes in sepsis, but can minimise pressure for antimicrobial resistance (AMR) by altering the way in which we use antibacterials in these very sick patients. Before addressing this question I will review, in this chapter, the definitions, epidemiology, aetiology and management of sepsis, with a focus on aetiology and antimicrobial treatment followed by the epidemiology and drivers of ESBL-E carriage, both with a focus on sSA.

1.2 Sepsis in sub-Saharan Africa

1.2.1 Search strategy

A review of the literature was undertaken to identify prospective cohort, case control studies or randomised controlled trials (RCTs) of sepsis in sub-Saharan Africa with the search terms shown in the appendix to this chapter. Pubmed and Scopus were searched, yielding 5460 unique studies on 17 July 2018. Inclusion criteria were any prospective cohort, RCT or case-control studies of sepsis in sSA (defined as taking place in the countries listed in search terms panel) recruiting patients using sepsis 1,2 or 3 definitions. Abstract review was undertaken resulting in inclusion of 91 studies for full text review. Eleven publications providing data on eight prospective cohorts[13–20] and three intervention studies (two RCTs[21,22] and one before-after intervention[23]) were identified. These data inform the following review, alongside non-systematically searched studies examining sepsis in high-resource settings.

In order to put sepsis aetiology data in context, systematic searches of the Pubmed and Scopus databases for leptospirosis, brucellosis, Q fever, Rickettsioses, arboviruses (dengue, or chikungunya) and histoplasmosis prevalence in unselected sepsis or fever cohorts in sSA were undertaken. Because a recent systematic review has examined these pathogens up to 2013 (see "sepsis aetiology" below), the date of these searches were restricted the 2014 to the present. Any studies examining disease prevalence in cohorts of febrile adults or children were included; outbreaks were excluded. Studies where the inclusion criteria were not clear (including those

with, for example, “suspected leptospirosis” with no further details) were excluded. Finally, systematic searches of *Pneumocystis jiroveci* pneumonia (PCP) were made using the search terms below; because a recent systematic review has examined the role of PCP up to 2015, the date on this search was restricted to 2015 or later. Table 1.1 shows the search terms, number of of hits and number of included studies after full text review: nine studies provided data on Leptospirosis[24–32], seven on Brucellosis[33–39], seven on Q-fever[31,35,40–43] , six on Rickettsioses[31,40,44–47], eighteen on Dengue[25,27,31,32,40,46,48–59], thirteen on Chikingunya[27,32,46,49,52,54,56–62], three on Zika [55–57], two on Histoplasmosis[63,64] and none on PCP. Details of the included studies are provided below.

Table 1.1: Search terms for fever studies

Organism	Search	n abstracts	n included
Leprospirosis	Leptospir AND	187	9
Brucellosis	Brucell AND	123	7
Q-fever	((Q fever) OR (coxiella)) AND	315	7
Rickettsioses	(Ricketts OR typhus OR (spotted fever)) AND	375	6
Arboviruses	(dengue OR chikungunya OR arbovir) AND	1422	Dengue 18, Chikungunya 13, Zika 3
Histoplasmosis	Histoplasm AND	72	2
PCP	(((((PCP) OR pneumocystis) OR "pneumocystis carini*") OR "pneumocystis jiroveci")) AND	87	0

Note:
All searches included the sSA country list in addition to the disease-specific terms above.

1.2.2 Statistical methods

Largely, narrative review of identified sepsis cohorts was undertaken, but meta analysis was used to summarise outcomes. 28 or 30-day sepsis mortality were extracted from identified studies or, if 28- or 30-day data were not available, in-hospital mortality was used. For interventional studies, in order to reflect the “usual-care” mortality, only the usual care arms were included. Pooled mortality estimates were then generated using a random effect meta-analysis of proportions with a generalised linear mixed model (GLMM, the so called binomial-normal model) using the *meta* package v.4.9.5[65] in R v3.6.0. Exact binomial 95% mortality confidence intervals were used throughout.

1.2.3 Defining sepsis

Sepsis is a heterogeneous syndrome, with no diagnostic gold standard. In 1991 the first modern sepsis diagnostic criteria were defined in a consensus conference of key opinion makers[66] (Table 1.8, chapter appendix). Sepsis was defined as the presence of the systemic inflammatory response syndrome (SIRS) plus infection, with a gradient of severity increasing through severe sepsis (sepsis plus organ dysfunction) to septic shock. These definitions were widely adopted as entry points into clinical trials, but ongoing concerns that SIRS was both insensitive and non-specific for the diagnosis of sepsis led to an expansion of the diagnostic criteria in 2001[67] again by expert consensus. Despite these revised guidelines the SIRS criteria largely continued to be preferred both as the entry point to clinical trials of sepsis and in clinical practice until the development of the current sepsis-3 definitions in 2016[2].

The sepsis-3 definitions redefined sepsis as "life threatening organ dysfunction triggered by infection", a definition that rendered the sepsis-2 severe sepsis category obsolete. In contrast to the previous diagnostic criteria that had relied largely on expert opinion, the sepsis-3 criteria attempted to use a probabilistic approach to defining sepsis, by mandating that sepsis should be associated with excess mortality. The sequential organ dysfunction score (SOFA, Table 1.9, chapter appendix), an organ-dysfunction score already in use in high income settings, and shown to be associated with mortality[68] was selected to operationalise the definition of sepsis. An acute change in SOFA of 2 or more points defines sepsis under sepsis-3.

Mindful that the SOFA score requires a large number of variables and is difficult to apply at the bedside, the consensus guideline group suggest the use of a simpler score, quick SOFA to identify patients who may have sepsis. Any two of: altered mental status, SBP < 100mmHg or respiratory rate > 22 breaths min^{-1} defines a positive qSOFA score. qSOFA does not define sepsis; rather, under sepsis-3 patients with a qSOFA score of 2 or more are at increased risk of poor outcomes and should be screened for sepsis using a full SOFA score. The qSOFA was derived by identifying factors associated with mortality in large datasets of patients with infection from the United States and validated in further US and German datasets; in these datasets it showed good discriminant ability to predict mortality, equivalent to full SOFA score outside the intensive therapy unit (ITU)[69].

Finally, sepsis-3 defines septic shock as persistent hypotension requiring vasopressors to maintain mean arterial blood pressure (MAP) above 65mmHg and serum lactate greater than 2mmol L^{-1}. This definition was arrived at by a combination of consensus and systematic review to identify potential defining variables and validation in large datasets from the United States, where it was found to be strongly associated with mortality[70].

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

Application of the sepsis-3 definitions, both in terms of clinical use and as inclusion criteria for research studies in sub-Saharan African low resource settings, is problematic. Several of the domains of SOFA require the results of blood tests, which may not be available. In Blantyre, and elsewhere in sSA, intensive organ support with inotropes or mechanical ventilation (invasive or non-invasive) may not be available[71] or be difficult to access[72], yet use of these treatment modalities form components of the SOFA score. Both lactate measurement and inotropic support may be unavailable in some settings and yet these define septic shock. Five studies have validated the qSOFA score in sub-Saharan African settings[18,73–76] and found variable discriminant ability for mortality but it is not clear how this score should be deployed in this setting; no studies have been undertaken to link qSOFA score to clinical action, and it is not intended to define sepsis under sepsis-3. The optimal sepsis definitions (both clinical and for research) for sSA are therefore not clear.

1.2.5 Sepsis epidemiology in sub-Sahara Africa

1.2.5.1 Incidence

The changing case definition of sepsis over time hampers estimation of incidence even in high-income settings, furthermore sepsis is not included in global burden of disease estimates. Different methods of defining sepsis from disease registries can result in very different estimates[77], but a recent systematic review and meta-analysis of 27 studies from 9 high income countries found a recent population incidence rate of 437/100,000 person-years (95% CI 334-571) for sepsis and 270 (95% CI 176 – 412) for severe sepsis with an increasing incidence over time from 1979 to 2015[4]. Crudely extrapolating these estimates to the worldwide population would result in 20.7 million sepsis and 10.7 million severe sepsis cases a year, largely in low resource settings. However, no data are available from low or middle income settings and these estimates must be treated with caution.

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

In high-income settings, risk factors for sepsis have been identified, though once again changing definitions as well as a lack of large scale community based studies make it difficult to draw definitive conclusions. However, chronic diseases (including HIV) and immunosuppressive agents have been associated with increased sepsis incidence, as well as older age[78,79]. In the United States, male sex and black ethnicity (vs white) and poverty are associated with increased sepsis incidence and severity[80].

Though equivalent studies aiming to identify risk factors for sepsis in adults in sSA are lacking, it is clear from the available data that HIV-infection is the dominant risk factor there. Summary patient demographics from the 10 identified sepsis studies are shown in Table 1.2; of 2788 included patients with available HIV status, 69% (1809/2788) were HIV infected, and often with advanced disease; of 1278 HIV-infected patients from 5 studies the study median CD4 count ranges from 52-168 cells/μL. In keeping with the epidemiology of the HIV epidemic in Africa, these patients are young, with average ages (variably reported as mean or median) ranging from 30-39 across the studies. These studies recruited an equal proportion of males and females (1444/2812 males, 51%), suggesting that sex is not a risk factor.

These data contrast sharply with the sepsis population in high income settings, from whom the majority of sepsis data have been generated, and who are older and mostly HIV uninfected[78,81,82]. The need for data from sSA to guide sepsis treatment protocols, rather than extrapolating from the high-income setting sepsis population, is clear.

Table 1.2: Characteristics of patients recruited to sSA sepsis studies

Study	Type	Year	Country	Inc. criteria	n	Male	Age	HIV infected	Median CD4
Jacob 2009	Cohort	2006	Uganda	Severe sepsis	382	156/382 (41%)	34.8 (11.2)	320/382 (85%)	52 (16-131)
Jacob 2012	Before-after	2006	Uganda	Severe sepsisc	245	95/245 (39%)	34 (28-41)	207/245 (86%)	43 (11-178)
		2008-09			426	207/426 (49%)	34 (27-40)	362/426 (85%)	63 (15-178)
Waitt 2015	Cohort	2008-09	Malawi	Sepsis	213	87/213 (41%)	30 (25-39)	161/213 (76%)	NR
Ssekitoleko 2011 (1)	Cohort	2009	Uganda	Sepsis	96	193/418 (46%)	35.1 (12.0)	331/418b (83%)	NR
Ssekitoleko 2011 (2)	Cohort	2009	Uganda	Sepsis	150	94/150 (63%)	35 (13)	96/150 (64%)	NR
Chimese 2012	Cohort	2010	Zambia	Sepsis	161	79/161 (49%)	39 (15.6)	110/138 (80%)	NR
Andrews 2014	RCT	2012	Zambia	Severe sepsis	112	58/109 (53%)	35 (1.4)	88/109 (81%)	NR
Auma 2013	Cohort	2012	Uganda	Sepsis	216	106/216 (49%)	32 (27-43)	122/216 (56%)	NR
Andrews 2017	RCT	2012-13	Zambia	Severe sepsis	209	117/209 (56%)	36.7 (12.4)	187/209 (89.5%)	66 (21-143)
Huson 2014	Cohort	2012-13	Gabon	Sepsis	384	142/382 (37%)	34 (25-46)	77/384 (20%)	168 (61-438)
Amir 2016	Cohort	2014-15	Uganda	Severe sepsis	218	110/218 (50%)	35 (26-50)	125/218 (57%)	78 (20-202)

Note:
RCT = randomised controlled trial. All studies use a modified sepsis-2 definition of sepsis or severe sepsis. Age is given as median (IQR) or mean (SD). Units of CD4 count are cells/microlitre. Jacob 2012 includes two cohorts of patients – results shown for both separately - and includes data from patients included in Jacob 2009. The n here includes those not included in this publication but the summary estimates include all patients as they cannot be disaggregated

1.2.5.3 Outcomes

Summary 28/30 day mortality outcomes for sepsis and severe sepsis in sSA from the identified studies are presented in Figure 1.1. It is clear that there is significant heterogeneity in outcomes of sepsis and severe sepsis in sSA, likely reflecting diverse patient and pathogen populations and variation in availability of available resources. This heterogeneity means that summary estimates should be interpreted with extreme caution but severe sepsis (49% [95% CI 39-58]), as expected, seems to carry a higher mortality hazard than sepsis (23% [95% CI 12-38]). Data of outcomes beyond 30 days are absent.

How does this compare to high income settings? A recent meta-analysis of population level estimates in high income settings found that a pooled sepsis 30-day mortality estimate of 17% (95% CI 11-26%)[4], though even older cohort studies as well as the more recent large sepsis-3 derivation cohorts have found considerably lower mortalities for sepsis (as defined by sepsis-2) ranging from 4-7%[69,83,84]. Most recent (largely post-2005) estimates of 30-day mortality from severe sepsis range from from 18-29%[4,5,77,82,85]. It seems likely therefore, that both sepsis and severe sepsis 30-day mortality is considerably higher in sSA than in high-income settings.

In the longer term, sepsis mortality continues to rise after the usual sepsis-study primary end point of 28 or 30 days, though data from sSA are absent. A systematic review in 2010 of long term sepsis mortality identified 26 studies (with none from low-resource settings) that reported long term sepsis mortality; 1 year mortality ranged from 22-72%, increasing to 45-75% at greater than 3 years[86]. Both short and long term morbidity is formidable also, though, once again, data from low income settings including sSA are absent[87–91] and health-related quality of life in sepsis survivors in high-income settings have been found to be persistently below population norms[86]. Long term sepsis outcomes in sSA are unknown.

1.2.6 Sepsis aetiology in sub-Saharan Africa

The 11 identified prospective sepsis studies in sSA carried out various combinations of diagnostic testing for malaria (either microscopy or rapid diagnostic test) and aerobic and mycobacterial blood culture; a summary is shown in Table 1.3 and 1.4. The commonest bloodstream infection (BSI) in all studies where mycobacterial blood cultures were carried out was tuberculosis – present in a higher proportion than of all BSI isolates from aerobic culture combined - though it is important to note that mycobacterial blood cultures in most studies were carried out in HIV infected people and bacteraemic tuberculosis was almost exclusively HIV-associated. With the exception of one study, malaria was less common than

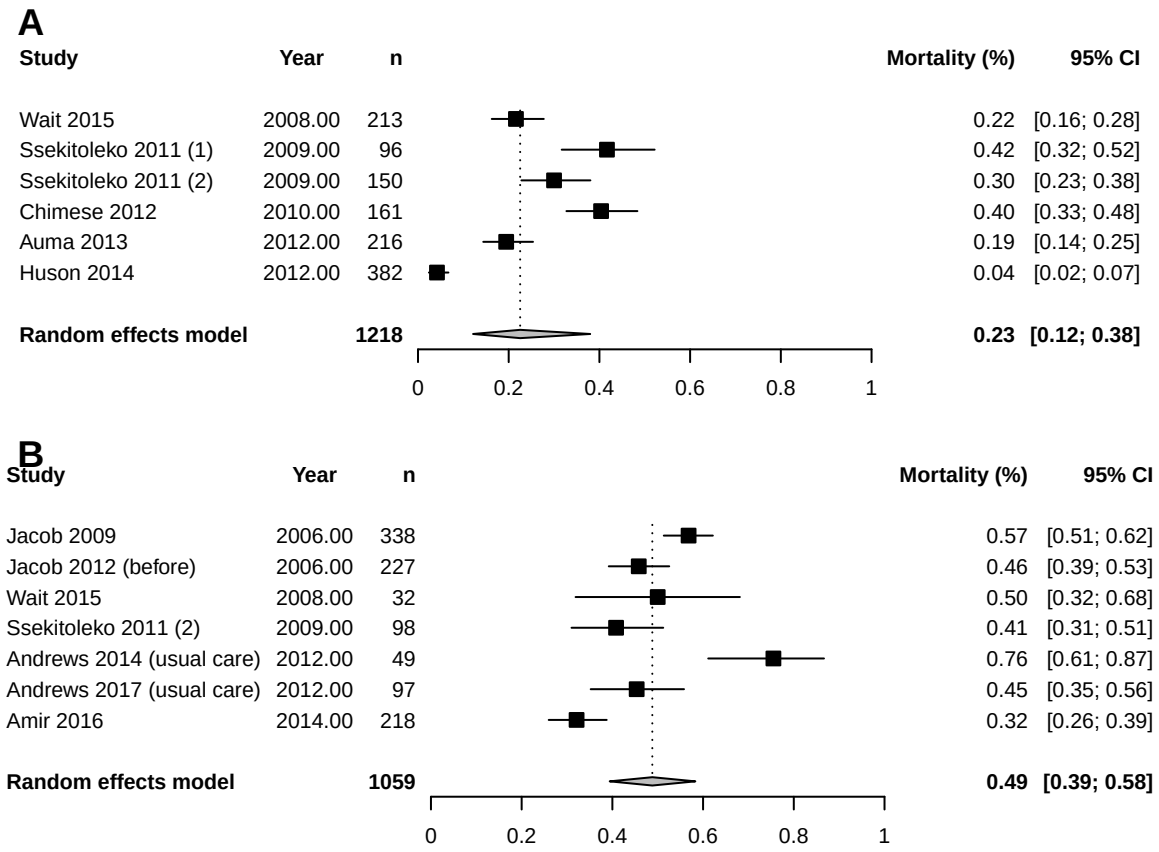

Figure 1.1: Pooled sepsis (A, top) and severe sepsis (B, bottom) inpatient mortality in sSA

BSI, highlighting the importance of non-malarial fever in sSA as malaria control efforts reduce the burden of malaria.

Table 1.3: Aetiology of sepsis in sSA

Study	BSI	MTB BSI	Malaria
Jacob 2009	48/382 (13%)	156/382 (22%)	34.8 (15%)
Jacob 2012	83/671 (12%)	104/576 (18%)	83/671 (12%)
Waitt 2015	33/213 (15%)	ND	26/213 (12%)
Ssekitoleko 2011 (1)	ND	ND	ND
Ssekitoleko 2011 (2)	39/150 (26%)	ND	7/150 (5%)
Chimese 2012	27/161 (17%)	ND	ND
Andrews 2014	26/109 (24%)	32/81 (40%)	2/109 (2%)
Auma 2013	41/216 (19%)	ND	9/216 (4%)
Andrews 2017	29/209 (14%)	43/187 (23%)	3/47 (6%)
Huson 2014	39/384 (10%)	NR	130/384 (33%)
Amir 2016	ND	ND	ND
TOTAL	**365/2493 (15%)**	**234/1093 (21%)**	**311/2139 (15%)**

Table 1.4: BSI isolates in sepsis in sSA

Organism	N
S. aureus	109
Non-Typhoidal Salmonellae	84
S. pneumoniae	67
Non-salmonella Enterobacteriaceae	46
Cryptococcus spp.	20
S. Typhi	6
Other	33
TOTAL	**365**

Note:
Excluded are coagulase-negative Staphylococci, alpha-haemolytic Streptococci other than Pneumococcus, *Bacillus* spp. and Micrococci as likely contaminants.

1.2.6.1 Tuberculosis

Beyond the studies of sepsis described above, there is ample evidence that disseminated tuberculosis is a significant cause of hospitalisation and death in people living with HIV in sSA, but optimum management in the context of critical illness is less clear. Mycobacterial blood culture is a difficult diagnostic tool to use in clinical practice - it requires laboratory infrastructure and usually takes many weeks to become positive - but when carried out hospitalised adults in sSA the prevalence of *Mycobacterium tuberculosis* bloodstream infection (MTB BSI) is between 2.5-23%, and almost universally restricted to participants with HIV[[92];[93]; [94];[95]; [96]; [97];[98]; [99]; [100]; [101]; [102];[103]; F@easey2013; [104]; W@addell2001].

The recent STAMP study in Malawi and South Africa found a mortality benefit in some prespecified subgroups of a strategy of screening all HIV-infected inpatients for TB using urinary lipoarabinomannan[105]. Autopsy studies have persistently found evidence of TB in a significant proportion of HIV-infected people who die in hospital - 43% in one meta analysis - which is often missed ante mortem, and is very often disseminated[106]. Both of these findings strongly suggest a high burden of undiagnosed disseminated TB in HIV-infected inpatients. The WHO, recognising this, has published guidelines on the management of smear negative tuberculosis in the seriously unwell[107] which suggest a trial of broad-spectrum antimicrobials for 3-5 days and prompt initiation of TB therapy if there is no response. Though based on expert opinion, these guidelines have been shown to improve outcomes in South Africa[108]. It is unknown whether delaying TB therapy in this way is associated with higher mortality in the critically unwell, analogously to antibacterial delay in sepsis in high income settings.

1.2.6.2 Bacterial zoonoses, Rickettsioses and arboviruses

There are several reasons to suspect that aetiologic agents other than bacterial BSI and tuberculosis may be significant in sSA, though data in sepsis are sparse. Studies of febrile illness in sSA have implicated Rickettsioses, arboviruses and bacterial zoonoses as causes of fever, accounting for a third of fever in hospitalised adults in one study in Tanzania[99]. Historically, however, data on these pathogens have been lacking. A 2015 systematic review of fever aetiology in LMIC (considering studies from 1980-2013) found that small numbers of patients had been systematically screened for these pathogens: in sSA 40/453 (8.8%) of adults with fever fulfilled diagnostic criteria for Leptospirosis, 16/453 (3.5%) for Brucellosis, 36/450 (8.0%) for spotted fever group Rickettsiosis, 24/482 (5%) for Q-fever and 55/700 (7.9%) for Chikingunya[10].

Increasing interest in non-malarial fever, however, has meant that data are accumulating from different settings in sSA, post-2013, as identified by the systematic review of the literature performed for this thesis. Details of the studies identified from this review are shown in Table 1.10 in the chapter appendix. These data highlight, firstly, the heterogeneity in diagnostics which are used for these pathogens – a combination of serology, PCR and antigen testing (often not using gold-standard case definitions), and secondly, the spatial and temporal heterogeneity across the continent.

These studies also demonstrate an increase, post-2013, in the use of molecular tests, particularly multiplex PCR assays (TaqMan array cards or PCR macroarrays) to detect multiple pathogens in fever aetiology studies. Despite the attractiveness of these assays – the ability to detect tens of pathogens in one assay on one body fluid sample – many infections will have only transiently detectable pathogen genetic material in blood and as such may have limited sensitivity. The

post-2013 fever aetiology data strongly suggest paired sera will maximise the diagnostic yield of bacterial zoonoses and Rickettsioses: for example, in studies of leptospirosis using PCR only 23/2533 (0.9%) of samples were positive versus 75/1464 (5.1%) in studies using paired sera; for Q-fever 9/3811 (0.2%) of samples were positive in PCR only studies versus 25/370 (6.8%) for paired sera studies; for Brucellosis PCR only studies 15/1005 (1.5%) of samples were positive versus 39/562 (6.9%) for paired sera studies; and for Rickettsioses 55/1932 (2.8%) of samples were positive for PCR studies vs paired sera 63/364 (17%). Some care must be taken with this conclusion: there are no studies that aim to directly compare paired sera and PCR assays for diagnosis of febrile illness, so the possibility of confounding remains.

Available data therefore suggest that bacterial zoonoses, Rickettsioses and arboviruses are significant causes of febrile illness in sSA. Their role is sepsis however is unknown. Only two studies have directly addressed the question of sepsis aetiology beyond BSI, malaria and TB: the first[9] performed PCR for 43 pathogens (using a TaqMan array card) including viruses (including dengue, chikungunya, and causes of viral haemorrhagic fever), bacteria (including *S. pneumoniae*, *E. coli*, *Salmonella* spp., *S. aureus* as well as *Coxiella burnetti*, *Rickettsia* spp., *Brucella* spp. and *Leptrospira* spp.), Mycobacterial (including *M. tuberculosis* (MTB) and *M. avium* complex), fungal (*Cryptococcus* and *Histoplasma spp.*) and parasitic (including malaria) on a convenience sample of 336 stored plasma samples from a Ugandan sepsis study. In keeping with the original study, MTB was frequently identified as was pneumococcus and malaria. Cytomegalovirus (CMV) was detected in 139/336 (41%) of patients, and was found to be independently associated with death, a finding which has been seen in sepsis studies in high-income settings[109] and may be related to the immune paresis of sepsis and CMV viraemia rather than disease. This study had no pathologic specimens and could not address this question. Dengue was detected in 17/336 (5%) of patients; *Rickettsia* spp. in 6/336 (2%), *Leptospira* spp. in 2/366 (0.6%) and *Coxiella burnetti* and *Brucella* spp. in 1/336 (0.3%) each. The true burden of disease of these pathogens may be higher, given the potential for increased diagnostic yield from serological assays.

The second study[110] is a retrospective analysis of a fever aetiology cohort from Tanzania, in which paired serology for bacterial zoonoses and Rickettsioses was carried out, as well as arboviral PCR. Of 423 enrolled adults, 25 were retrospectively classified as having septic shock, 37 severe respiratory distress without shock and 109 severe pneumonia by WHO Integrated Management of Adolescent and Adult Illness (IMAI) District Clinician Manual criteria[111]. These patients would likely fulfil sepsis criteria under sepsis-2 or 3 guidelines, and were found to have a variety of diagnoses, though not all patients had all diagnostic tests: Chikungunya (6/154 [3%]),Leptospirosis (5/82 [6%]), Coxiella burnetti (7/83 [8%]) and spotted fever group Rickettsioses (6/83 [7%]).

1.2.6.3 HIV opportunistic infections: PCP, histoplasmosis and cryptococcal disease

The burden of HIV opportunistic infections in sepsis in sSA (including PCP, cryptococcal disease and including here histoplasmosis as an opportunistic infection) is unclear. Beyond blood culture identification of *Cryptococcus neoformans* (present in 20/365 of positive blood cultures in the sepsis studies identified in this review) none of these pathogens have been systematically sought in sepsis cohorts in sSA, and their role as causative agents of sepsis is far from clear. Cryptococcal disease most commonly manifests as cryptococcal meningitis, is common in HIV infection and is thought to account globally for 15% of AIDS-related deaths[112]. No study has attempted to define the burden of PCP in sepsis in sSA, though a 2016 systematic review[113] addressed the prevalence and attributable mortality of PCP , finding the pooled prevalence of PCP in inpatients (n = 2593, 23 studies) to be 22% (90% CI 17 – 27%) in random effect meta-analysis. Data examining the role of Histoplasmosis as a cause of fever or sepsis in sSA are sparse. A 2015 systematic review[10] identified only one study up to 2013 which Histoplasma urine antigen testing in 628 febrile adults and children in Tanzania finding 9/628 (1%) probable cases, 6/9 of whom were HIV infected. Since then one study in Uganda found 0/151 HIV-infected patients with suspected meningitis[63] had detectable IgM to *Histoplasma capsulatum* and and no Histoplasma antigen was detected in serum (n = 57), urine (n = 37) or CSF (n=63); a study in Cameroon[64] used histopathologic examination and culture to identify histoplasmosis in 7/56 (13%) of HIV infected patients with CD4 < 200 cellsμL^{-1} and chronic cough with histoplasmosis like skin manifestations.

1.2.7 Sepsis management

The cornerstone of sepsis management is rapid administration of appropriate antimicrobial therapy, source control of any infectious focus and normalisation of tissue perfusion using intravenous fluids and, if necessary, inotropes, with other organ support as necessary (e.g. intubation and mechanical ventilation and renal replacement therapy). Several international guidelines for sepsis care are available; this section will examine these and specific guidance for sepsis in adults in sSA followed by a review of the evidence to inform these guidelines.

The surviving sepsis campaign has published four editions of comprehensive guidance on the management of sepsis in adults, which are endorsed by all the major critical care organisation in high income settings and form the basis of most sepsis care in high income settings; selected major recommendations of the latest guidance[114] are shown in Table 1.5 below.

Table 1.5: Surviving sepsis campaign guidelines

Recommendation	Strength of recommendation	Quality of evidence
Resuscitation		
Administer 30ml/kg of intravenous crystalloid solution, within 3hr of diagnosis of sepsis	Strong	Low
Use frequent reassessment to guide further fluid	BPS	BPS
Use dynamic variables to assess fluid responsiveness (e.g. cardiac output)	Weak	Low
Use vasopressors in patients who remain hypotensive despite adequate fluid resuscitation; target a MAP of 65mmHg	Strong	Moderate
Use noradrenaline as first-line vasopressor	Strong	Moderate
Measure lactate and use lactate normalisation to guide resuscitation in patients with elevated lactate	Weak	Low
Antimicrobials		
Administer broad spectrum antibiotics within 1hr of diagnosis of sepsis	Strong	Moderate
Adjunctive therapies		
Use hydrocortisone 200mg IV per day if adequate fluid resuscitation and vasopressor therapy are unable to restore haemodynamic stability	Weak	Low

Note:
BPS = best practice statement

Mindful that guidelines aimed at high-income settings may be impossible to implement in low-resource settings (including large areas of sSA) the Global Intensive Care Working Group of the European Society of Intensive Care Medicine (ESICM) published recommendations for sepsis management in resource-limited settings in 2012[115], endorsed by a number of national and international sepsis organisations, and supplements in 2016-17 covering general supportive care[116], infection management[117], management of severe malaria and severe dengue[115] and haemodynamic assessment and support[118] in sepsis in low-resource settings. The major recommendations of this guidance are consolidated in Table 1.6 below.

Table 1.6: ESICM low resource setting sepsis recommendations

Recommendation	Strength of recommendation	Quality of evidence
Resuscitation		
Use capillary refill time, skin mottling scores or skin temperature gradients to assess adequacy of tissue perfusion.	Weak	Ungraded
Use passive leg raise (PLR) to guide fluid resuscitation in sepsis or septic shock	Weak	High
Use crystalloid for fluid resuscitation	Strong	Moderate
Give 30ml/kg of fluid over the first 3hr following sepsis diagnosis, to start within 30mins of recognition	Strong	High
Larger volumes of fluid may be needed if the patient remains fluid responsive and still shows signs of tissue hypoperfusion	Strong	Low
Be extremely cautious in settings with no or limited access to vasopressors and mechanical ventilation and consider stopping fluid if respiratory distress or lung crepitations develop	Strong	High
Use noradrenaline as first line vasopressor	Strong	Moderate
Target a MAP of > 65mmHg	Strong	Moderate
Antimicrobials		
Appropriate antibiotics should be given within the first our following septic shock	Strong	Low
Source control should occur within 12hr of admission to hospital	Ungraded	Ungraded
Adjunctive therapies		
Use hydrocortisone 200mg IV per day if adequate fluid resuscitation and vasopressor therapy are unable to restore haemodynamic stability	Weak	Low

Note:
MAP = Mean arterial blood pressure

The World Health Organisation (WHO) in 2011 published the integrated management of adolescent and adult illness (IMAI) guidance[111], which includes guidance on the management of septic shock and is aimed at district-level clinicians in resource limited settings rather than critical care clinicians. This suggests defining shock as SBP < 90mmHg or pulse > 110/minute and suggest that, once shock is identified, oxygen should be given, a 1 litre bolus of fluid should be given immediately and pulse, SBP and signs of perfusion (urine output, mental status) should be rechecked. If shock persists, another litre should be given; if shock persists after the second litre then help should be sought. Antimicrobials should be administered: ceftriaxone IV or IM, and antimalarials if indicated. No evidence base is referenced for these recommendations.

1.2.7.1 Early goal directed therapy

In 2001 a pivotal single centre study in the United States of 263 patients with severe sepsis or septic shock[119] found that protocolised aggressive early resuscitation (called Early Goal Directed Therapy, EGDT) significantly reduced mortality from 46.5% to 30.5%. EGDT called for early central venous catheterisation and protocolised resuscitation to central venous pressure (CVP), MAP and central venous oxygen saturation targets (ScvO2), and was widely adopted. However three large multicentre randomised controlled trials of EGDT – ProCESS in the United States[120], ARISE in Australasia[121] and ProMISe[122] in the United Kingdom, reporting in 2014 and 2015 failed to show any difference in outcomes between the EGDT and usual-care arms. A pre-planned individual level meta-analysis of the 3723 patients included in these trials confirmed similar 90 day mortality in both arms (24.9% for EGDT vs 25.4% for usual care, aOR 0.97 [95% CI 0.82-1.14]) with no benefit found in pre-planned subgroup analysis for patients with worse shock or in hospitals with lower propensity for vasopressors or fluid administration[82]. It is likely therefore that the tenets of EGDT that improve outcomes (early antimicrobials and aggressive fluid resuscitation) have been absorbed into usual care in the fifteen years since the original EGDT study, as evidenced by the reduction is sepsis mortality over this time period, and so the specific package of protocolised care and EGDT targets does not in itself improve outcomes. Unanswered questions now remain regarding the most effective use of the individual components of EGDT (fluids, vasopressors etc). A number of attempts have been made to develop protocolised sepsis care packages in the style of EGDT for sSA; these are described below in relation to the individual components of sepsis care.

1.2.7.2 Evidence to guide antimicrobial therapy in sSA

There is evidence from high income settings that delay in appropriate antimicrobial administration is associated with worse outcomes in sepsis. The first study to investigate this relationship, published in 2006, found a very strong relationship between time to appropriate antimicrobial administration from onset of hypotension and mortality with an absolute increase in mortality of 7.6% for each hour delay over the first six hours[123]. Subsequent data have been more nuanced: a 2015 meta-analysis addressing this question identified 11 studies of 16,178 patients and found no relationship between antimicrobial delay and mortality[124], though many of the included studies are open to confounding by indication (sicker patients are given antimicrobials more quickly), timed antimicrobial administration to non-physiological events (e.g. arrival to hospital or time of blood culture draw rather than onset of hypotension) and did not assess the appropriateness of antimicrobial therapy, all of which could mask a relationship. Appropriate antimicrobial therapy has certainly been shown to be associated with improved survival: a 2010 meta-analysis quantified the pooled adjusted odds ratio to be 1.6 (95% CI 1.4-1.9) from

26 studies for appropriate versus inappropriate antimicrobial therapy[Paul2010]. A recent large retrospective study of 49,331 patients in New York hospitals[81] confirmed the relationship between antimicrobial delay and mortality with an adjusted odds ratio of in-hospital death of 1.04 per hour delay (95% CI, 1.03 – 1.06), and rapid antimicrobial administration forms a key recommendation of current sepsis guidelines.

Data from sSA are lacking, however; neither of the meta analyses above (including between them 37 studies) included any data from sSA, but three of the sepsis studies identified in this systematic review attempt to address the question. The first[13], in an observational study of 382 adults with severe sepsis in Uganda found no association between administration of antibiotics within 1 hour and mortality (OR 0.9 [95% CI 0.6-1.6]) but a total of 42 antibiotic regimens were used and there was a high proportion (22%) of bacteraemic tuberculosis; no assessment of appropriateness of antimicrobials was undertaken and it is possible that inappropriate antimicrobials could mask any association between time of administration and mortality, if one existed.

The second[23], interventional, study in the same centres in Uganda used a before-after design with 661 patients to implement a clinical-officer delivered fluid resuscitation protocol (see below) and administration of antimicrobials. 426 patients were included in the intervention with 245 in the usual care group. The protocol resulted in more rapid administration of antibiotics (67% administered within 1hr versus 30%, $p < 0.001$) and less (though still very prevalent) inappropriate antimicrobial administration (81% versus 95%, $p < 0.001$). Antimicrobial administration was associated with a reduced hazard of death in a multivariable Cox proportional hazards model, but the comparator group used was patients who received no antimicrobials and the hazard ratio for rapid administration (< 1hr HR 0.44 [95% CI 0.21 – 0.89]) was not significantly different from delayed administration (> 6hr HR 0.39 [95% CI 0.19 – 0.81]). This type of study design is very prone to bias due to confounding as sepsis management changes over time, especially as the "before" arm was recruited two years before the "after" arm, so results from this study should be interpreted with caution.

A third observational study in a Ugandan teaching hospital[125] provides data on the effect of rapidity of administration of antimicrobials; this study enrolled 218 patients; 89% of them received any antibiotics within 6 hours, with a median time to antibiotic administration of 30mins. Antibiotic administration within 6hr (versus not) was not significantly associated with in hospital mortality in univariate analysis (OR 1.5 95% CI 0.6 – 3.8) though the confidence intervals are wide and could incorporate a clinically significant effect. Again, no assessment of appropriateness of antimicrobials was made.

Only one study provides limited evidence that appropriate antimicrobial therapy improves outcomes in infection in sSA[126]: a combined retrospective-prospective analysis of 104

patients with typhoid perforation (defined by clinical and operative findings rather than culture) from a single Tanzanian teaching hospital found that adequate antimicrobial exposure (defined as at least 3 days of antimicrobial active against S. Typhi prior to hospital admission) was associated with improved in-hospital survival in multivariable analysis (aOR 2.9 [95% CI 2.1-4.5]), however it is doubtful that this very specific complication of typhoid fever is generalizable.

1.2.7.3 Intravenous fluid therapy in sub-Saharan Africa

The evidence base for rapid fluid administration – and the surviving sepsis recommendation of 30ml/kg within 3hrs following diagnosis - is less secure than for rapid antimicrobial administration. As with antimicrobial administration, adoption of guidelines in response to the EGDT study has meant that disentangling the independent effect of fluid administration is difficult. The data are contradictory. Several large retrospective observational analyses have found no impact on rapidity of fluid bolus administration following sepsis diagnosis[81,127]. Indeed, fluid clearly has the potential for harm; positive fluid balance for patients with sepsis in the ITU has been persistently linked with worse outcomes[@[[81]; Boyd2011; Vincent2006]. Other studies, in contrast, have found that more rapid initiation of IV fluid is beneficial [128,129]. It may be that heterogeneity in response to fluids plays a role in these conflicting findings; a retrospective multicentre cohort analysis of 3686 patients[130] found that 64% were "fluid responders" – that is, they had a sustained blood pressure response to initial fluid resuscitation without need for vasopressors, and mortality was 15% greater (95% CI 10-18%) in fluid nonresponders.

In sSA, in some ways, the picture is clearer: there is increasing evidence that liberal intravenous fluid administration to septic patients causes harm. The landmark FEAST trial[8] randomised 3141 children with severe febrile illness in Kenya, Uganda and Tanzania to receive either albumin bolus or 0.9% saline bolus or usual care and found an increased risk of death by 48 hours in both bolus groups (RR 1.45 [95% CI 0.78-1.29] for any bolus compared to no bolus). In a secondary analysis[131] this was thought to be due to cardiovascular collapse rather than pulmonary oedema; the mechanism of this is unclear. Only three controlled studies have addressed the question of optimal intravenous fluid resuscitation for septic adults in sSA. One before-after intervention study in septic shock patients carried out in Uganda and described above[23], found a mortality benefit of $>$ 1L fluid over the first 6hr compared to $<$ 1L in multivariable Cox proportional hazard model (HR 0.54 [95% CI 0.35-0.82] 1.0 – 2.5 L vs $<$ 1.0L) but was hampered by a before-after design. Two randomised controlled trials of protocolised early sepsis care in adults have been carried out at a single centre in Zambia assessing the effect of a protocol that administers up to 4L of fluid over 6hrs. The first[21]

recruited patients with severe sepsis with organ dysfunction criteria including respiratory rate > 40/min and was stopped early as it was felt that participants with baseline respiratory compromise might be at risk of harm; 7/10 (80%) of this subgroup died in the usual care group, compared to 8/8 (100%) in the intervention group (p = 0.09). The second was unequivocally harmful, with more participants dying by 28 days in the intervention group (48% vs 33%, p = 0.03). The reasons are not clear. More respiratory compromise (defined as increase in respiratory rate by 5 breaths/min or reduction in oxygen saturation of 3% or more) occurred in the intervention group (35% vs 22%, p =0.03) but persisted beyond 6 hours in similar numbers in both groups (17% vs 15%, p = 0.63).

1.3 ESBL-E in sub-Saharan Africa

I here discuss the definition and global epidemiology of ESBL-E, with systematic review and meta analysis of the prevalence of ESBL-E colonisation and invasive infection.

1.3.1 Introduction: definition and classification of ESBL-E

β-lactamases are enzymes that hydrolyse the active β-lactam ring in β-lactam antimicrobials. Two classification schemes are usually used for β-lactamases: the molecular (or structural) classification of Ambler[132], or the Bush-Jacoby-Medeiros functional classification[133] (Table 1.7). Molecular classification is straightforward and depends on protein homology; class A, C and D enzymes are serine β-lactamases and class B are metallo-β-lactamases, named for the composition of their active site. The functional classification is complex and clusters enzymes into four groups, with a number of subgroups, based on substrates and the effect of β-lactamase inhibitors and EDTA: class 1 (corresponding to Ambler class C) are cephalosporinases that are not inhibited by clavulanic acid, and includes the AmpC enzymes of the Enterobacteriaceae; class 2 enzymes are β-lactamases that are largely inhibited by clavulanic acid and belong to Ambler class A or C; and class 3 are the metallo-β-lactamases corresponding to Ambler class B. Class 4 enzymes are penicillinases which are not inhibited by clavulanic acid, though are of limited significance and not included in Table 1.7.

The vast majority of clinically relevant ESBLs belong to Ambler class A, functional class 2be. For the purpose of this thesis, therefore, I define ESBL as enzymes which confer resistance via hydrolysis to penicillins, cephalosporins of the first, second or third generation (excluding cephamycins), aztreonam, but not carbapenems, and are inhibited by β-lactamase inhibitors such as clavulanic acid[134]; this corresponds to the Bush-Jacoby group 2be and makes clear that I draw a distinction between ESBL and AmpC enzymes, which would be grouped with the

Bush-Jacoby group 1. When referring to ESBL genes I will refer to them is the standard way using *bla* and a subscript to indicate the gene as for example $bla_{CTX-M-15}$; when referring to the enzyme, I will refer to them as, for example CTX-M-15.

Table 1.7: ESBL classification. Adapted from [133]

			Inhibited by			
Bush Jacoby group	Molecular class	Distinctive substrates	BLI	EDTA	Defining hydrolysis spectrum characteristics	Representative enzymes
1	C	Cephalosporins	No	No	cephalosporins > benpen, hydrolyzes cephamycins	E. coli AmpC, P99, ACT-1, CMY-2, FOX-1, MIR-1
1e	C	Cephalosporins	No	No	ceftazidime and often other oxyimino-beta-lactams	GC1, CMY-37
2a	A	Penicillins	Yes	No	benzylpen > cephalosporins	PC1
2b	A	Penicillins, early cephalosporins	Yes	No	Similar hydrolysis of benzylpenicillin, cephalosporins	TEM-1, TEM-2, SHV-1
2be	A	Extended-spectrum cephalosporins, monobactams	Yes	No	oxyimino-beta lactams	TEM-3, SHV-2, CTX-M-15, PER-1, VEB-1
2br	A	Penicillins	No	No	Resistance to BLI	TEM-30, SHV-10
2ber	A	Extended-spectrum cephalosporins, monobactams	No	No	oxyimino-beta lactams plus resistance to BLI	TEM-50
2c	A	Carbenicillin	Yes	No	Increased hydrolysis of carbenicillin	PSE-1, CARB-3
2ce	A	Carbenicillin, cefepime	Yes	No	Increased hydrolysis of carbenicillin, cefepime, and cefpirome	RTG-4
2d	D	Cloxacillin	Variable	No	Increased hydrolysis of cloxacillin or oxacillin	OXA-1, OXA-10
2de	D	Extended-spectrum cephalosporins	Variable	No	cloxacillin or oxacillin and oxyimino-beta-lactams	OXA-11, OXA-15
2df	D	Carbapenems	Variable	No	cloxacillin or oxacillin and carbapenems	OXA-23, OXA-48

Table 1.7: ESBL classification. Adapted from [133] *(continued)*

Bush Jacoby group	Molecular class	Distinctive substrates	BLI	EDTA	Defining hydrolysis spectrum characteristics	Representative enzymes
2e	A	Extended-spectrum cephalosporins	Yes	No	Inhibited by clavulanic acid but not aztreonam	CepA
2f	A	Carbapenems	Variable	No	carbapenems, oxyimino-beta-lactams, cephamycins	KPC-2, IMI-1, SME-1
3a	B (B1)	Carbapenems	No	Yes	includes carbapenems but not monobactams	IMP-1, VIM-1,CcrA, IND-1
	B (B3)					L1, CAU-1, GOB-1, FEZ-1
3b	B (B2)	Carbapenems	No	Yes	Preferential hydrolysis of carbapenems	CphA, Sfh-1

Note:
BLI = Beta-lactamase inhibitor

1.3.2 Global molecular epidemiology of ESBL-E: an overview

The history of the global spread of ESBL-E is complex and an enormous number of unique ESBL amino acid sequences have been described; at the time of writing the NCBI beta-lactamase directory contains 1557 named β-lactamase genes, many of them ESBL. However, there are 3 families of enzyme which cause the majority of infections in humans: TEM}, SHV}, and CTX-M. They will be briefly described here in turn in the context of their putative origins and global dissemination in the latter half of the 20th century. A diverse range of other ESBL enzymes have been described, but are largely of less clinical significance than those described above, and are beyond the scope of this review: most notably the OXA type, which in contrast to TEM, SHV, and CTX-M, are of the molecular class D and functional class 2d, and are characterised by a high rate of hydrolysis of cloxacillin[135]; like TEM and SHV, OXA beta-lactamases are not always extended-spectrum.

1.3.2.1 1980s-1990s: First identification of ESBL in nosocomial pathogens

β-lactamases form an integral part of the natural armamentarium of many genera of bacteria – particularly gram negatives, including Enterobacteriaceae - and predate the antibiotic era; penicillinases were identified in *E. coli*, for example, prior to the widespread introduction

of penicillin for treatment of human disease[136]. These β-lactamases are often chromosomally located; the first plasmid-mediated narrow-spectrum β-lactamase, TEM-1 -named for the patient, Temoneira, from whose blood it was first isolated – was found in Athens in the 1960s[137]. It rapidly disseminated globally and is thought to be responsible for a high proportion of ampicillin resistance in *E. coli*[135]. This worldwide spread spurred the development and use of β-lactamase resistant extended-spectrum cephalosporin antimicrobials, which found wide use in the 1980s. Perhaps inevitably, an enzyme conferring resistance to extended-spectrum oxyimino-cephalosporins was subsequently identified in a German clinical *Klebsiella ozaenae* isolate in 1983, encoded for on a pBP60 plasmid and enzymes of this sort were named ESBLs[138,139].

This first ESBL enzyme was found to be similar to an existing plasmid-encoded narrow spectrum beta lactamase, SHV-1, which had been described in the 1970s in *E. coli*, and was thought to itself be descended from a chromosomally encoded *K. pnemoniae* narrow spectrum beta lactamase which was liberated onto a plasmid[140]. The point mutations in SHV-1 conferred the ESBL phenotype, and this enzyme was named SHV-2. This pattern - mutation of a narrow spectrum β-lactamase to produce an ESBL phenotype - also occurred in TEM, and the first ESBL TEM was described in France in 1989[141] and named TEM-3. Many TEM and SHV variants were subsequently described[142]. However, in this early stage of the epidemic, ESBL enzymes were largely nosocomial, and often associated with *Klebsiella spp.*[143].

1.3.2.2 1990s-2010s: Emergence and globalisation of CTX-M

From the late 1990s onwards, there were profound changes in the global epidemiology of ESBL-E, on three fronts, all intricately interrelated, and occurring simultaneously: first, the rapid emergence and globalisation of the successful CTX-M ESBL enzyme family[144], aided by mobile genetic elements; second, *E. coli* joining *Klebsiella spp.* as a major ESBL host[145], and the emergence of so-called high risk bacterial clones; and third, the spread of ESBL-E into the community[146]. CTX-M-1 was first identified and named in Germany in 1989[147], the name derived from "active on cefotaxime first isolated in Munich." Many variants were subsequently identified, largely in *E. coli* and *K. pneumoniae*, from isolates all over the world[148]. The bla_{CTX-M} genes are clustered by homology into 5 groups (bla_{CTX-M} groups 1,2,8,9 and 25) and each group is thought to have descended from a chromosomal beta lactamase from *Kluyvera spp.*[144]

A year-on-year rise in incidence of invasive ESBL-E infection was seen in most high-income settings (Figure 1.2) throughout the 2000s and 2010s, the majority of which were CTX-M producers, though with varying proportions of different CTX-M enzymes in different

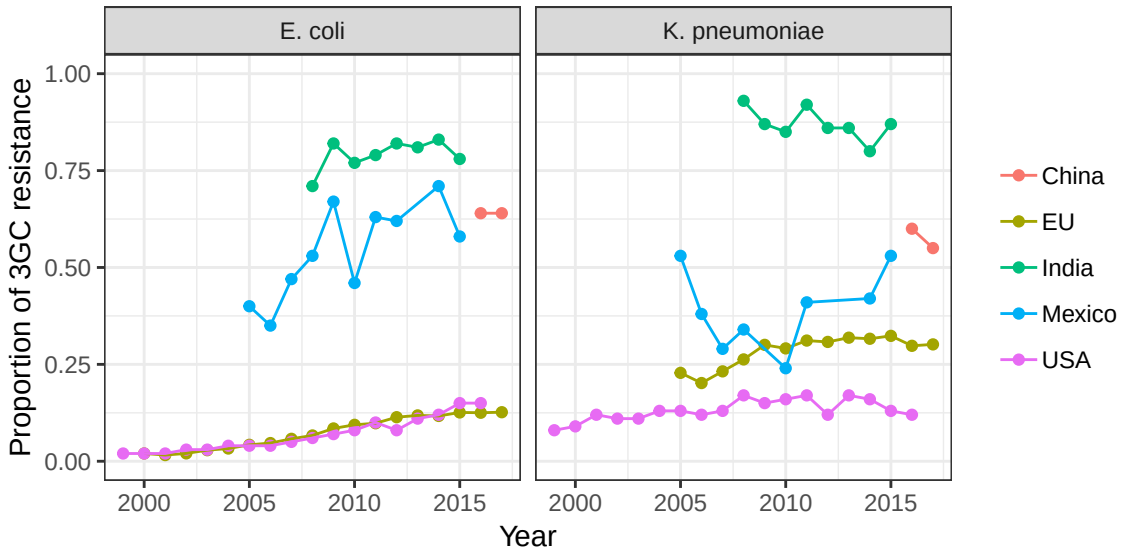

Figure 1.2: Prevalence of third generation cephalosporin resistance in representative high income (EU, USA) and middle income (China, Mexico, India) areas in invasive E. coli (left panel) and K. pneumoniae (right panel) isolates. Data for EU are from ECDC surveillance atlas (https://ecdc.europa.eu/en/antimicrobial-resistance/surveillance-and-disease-data/data-ecdc) and for other countries are from CCDEP resistance map (https://resistancemap.cddep.org/AntibioticResistance.php) both accessed 28 December 2018. 3GC = third generation cephalosporin. Note that these data are 3GC-resistant isolates rather than confirmed ESBL-producers, but would be expected to be ESBL-producers in the vast majority of cases.

locations[145,149]. Risk factors for ESBL-E infection in high income settings have persistently been shown to be hospital or long-term care facility exposure, antimicrobial exposure and chronic health conditions though it was recognised in the 2000s that a large proportion of patients with invasive ESBL-E lack any of these risk factors[146], suggesting acquisition in the community. Colonisation prior to infection is thought to be the norm; prior colonisation is a significant risk factor for infection and indeed when sought ESBL-E are found in the stool of healthy community members worldwide[150].

Though less comprehensive, data from middle income countries suggests that prevalence of ESBL producers amongst invasive *E. coli* and *K. pneumoniae* are very high (Figure 1.2) and in countries such as India invasive *E. coli* and *K. pneumoniae* that are sensitive to third-generation cephalosporins are in the minority. The reasons for this are not clear but country and regional level associations (which are open to ecologic bias) have been shown with antimicrobial consumption[151,152] and economic status; GDP per capita has been found to correlate inversely at a country level with third-generation cephalosporin resistance rates[153]. Data from sSA have historically been lacking and are systematically reviewed below.

1.3.2.3 Epidemiology of gut mucosal carriage of ESBL-E: the first step towards invasive infection

Invasive infections with Enterobacteriaceae are thought to usually result from infection from an individual's own gut microbiota, irrespective of resistance pattern[154], and whole genome sequencing has confirmed that invasive isolates are often closely related to prior gut carriage isolates[155]. Strategies to minimise carriage are therefore potentially attractive as interventions to reduce invasive infection and a number of studies have attempted to understand the dynamics of gut mucosal ESBL-E carriage in health and disease. A brief overview of ESBL-E community carriage is presented here, and ESBL-E carriage in sSA is systematically reviewed below.

ESBL-E community carriage in Spanish outpatients[156] and healthy children in Poland[157] was first described in 2001. Since that time carriage by healthy community members has been found worldwide in all populations[150,158], though there are heterogeneities between and within countries which mirror the prevalence of invasive ESBL-E prevalence amongst *E. coli* and *K. pneumoniae*. In Europe, for example, community prevalence of ESBL-E carriage was estimated be 7.3% in the UK in 2014 in a large community study[159], 4.5% in the Netherlands in 2012[160] and 4.7% in Sweden in 2012/13[161] and 3.7% in Spain in 2003[162], significantly lower than community carriage prevalence of 50.9% seen in China in 2009[163] or 33.8% in India in 2011-2013[164].

Risk factors for colonisation have been identified in many studies and antimicrobial exposure[165,166] and healthcare facility exposure[[164]; Luvsansharav2012] (including long term care facilities[167]) are consistently identified as such. Colonisation of a household member has also been identified as a risk factor[[168]; Rodriguez-Bano2008], suggesting significant within-household spread. Antacid use has been associated with ESBL-E colonisation[165] as has exposure to farming[@[160]. In low prevalence areas, travel to high prevalence areas is a risk factor[159,161,165,166,169].

The majority of studies of ESBL-E carriage are cross sectional and only a handful have attempted to characterise longitudinal carriage of ESBL-E with a longitudinal sampling approach. Estimates of carriage duration vary, partly because of the difficulty in inferring them from interval-censored rectal swab or stool data, but it is clear that some patients remain colonised for many months. Following a Swedish ESBL-E outbreak, 12% of patients still carried ESBL-E at the final sampling visit, a median 58 months after the outbreak[170]. French and German studies found a median duration of carriage of 4.3[171] and 12.5[172] months respectively following hospitalisation or outbreak. More transient carriage following international travel seems to be the norm with a median of 30 days in a large Dutch study[173];

the reasons for this are not clear.

The largest longitudinal community study of ESBL-E carriage took place in the Netherlands which recruited 76 ESBL-E colonised and 249 uncolonised community members and carried out longitudinal stool sampling at 5 time points over 8 months. 25/76 (32.9%) of initially-colonised participants remained persistently colonised after a median 242 days. Antimicrobial exposure in the past 6 months, proton-pump inhibitor use, colonisation with *E. coli* phylogroup B2 or D and presence of $bla_{CTX-M-27}$ or $bla_{CTX-M-14}$ was associated with persistent carriage, suggesting both host and bacterial factors may be important determinants of carriage duration. *K. pneumoniae* colonisation seemed to be less common in the persistent carriage group[169,174]. This study also found significant heterogeneity of *E.coli* sequence type in longitudinal samples of persistent carriers but that ESBL genes and often detectable plasmid replicons remained unchanged, suggesting a significant role for mobile genetic elements.

1.3.2.4 Molecular mechanisms underlying success of CTX-M: mobile genetic elements and high-risk clones

The remarkable success of CTX-M has led to efforts to understand the molecular mechanisms by which this enzyme spread so rapidly. The system is complex, and poorly understood, but should be considered at multiple levels including that of the organism; the plasmid; the transposon, which may contain integrons or insertion sequences and, at the lowest level the ESBL gene. These will briefly be reviewed here.

The initial mobilisation event of bla_{CTX-M} from *Kluyvera spp.* is thought to have been mediated by capture of transposable insertion sequences; the insertion sequence *ISEcp1* has been experimentally demonstrated to mobilise the bla_{CTX-M} precursor from *Kluyvera ascorbata*[175] and *ISEcp1* is most consistently associated with bla_{CTX-M} genes but *IS26*, *ISCR1* and *IS10* have also persistently been described upstream from bla_{CTX-M} genes, suggesting multiple mobilisation events[149]. There is also an association between particular pairs of bla_{CTX-M} gene clusters and insertion sequences, consistent with a hypothesis of multiple mobilisation events[176]. These insertion sequences provide two roles: they encode a transposase enabling gene mobilisation but act as a strong promotor of bla_{CTX-M}, without which phenotypic cephalosporin resistance is absent or reduced[177].

After mobilisation from the *Kluyvera* genome, the bla_{CTX-M} genes were integrated onto a plasmid backbone, a process which is likely ongoing as a substantial number of diverse bla_{CTX-M} carrying plasmids have been described: there is, however, an association between bla_{CTX-M} genotype and plasmid incompatibility group. The successful $bla_{CTX-M-15}$ gene is very strongly associated with the narrow host-range IncF plasmid group, for example, which

are restricted to Enterobacteriaceae[[177]; Carattoli2009]. Identical bla_{CTX-M} containing plasmids have been found across diverse geographical regions and have been termed "epidemic plasmids"[149] though the mechanism of persistence of these plasmids within a bacterial population remains unclear.

In addition to frequently co-occurring bla_{CTX-M} genes, transposable elements and plasmids, some clonal groups of *E. coli* and *K. pneumoniae* are both globally successful and associated with particular bla_{CTX-M} genes and plasmids. These successful sequence types (STs) are known as "high risk clones." The archetypal example is *E. Coli* ST131 which is often associated with an IncFII plasmid containing $bla_{CTX-M-15}$[178]. First described in 2008, *E. coli* ST131 is thought to be responsible for around 80% of extra-intestinal ESBL *E. coli* infection[179]. Population genomics studies have demonstrated that a particular clade, ST131 clade C, is globally dominant and have shown a sequential acquisition of virulence determinants followed by mobile genetic elements conferring fluoroquinolone and ESBL resistance[180,181]. These events may have contributed to the global success of ST131, but the precise mechanism of its apparent fitness advantage remains unknown.

1.3.3 Epidemiology of ESBL-E in sub-Saharan Africa

In order to clearly define the epidemiology of ESBL-E in sSA I performed a systematic review and meta analysis, which is presented here.

1.3.3.1 Search strategy

A systematic review of the literature was undertaken to answer the following questions: firstly, what is the prevalence of ESBL-E amongst invasive isolates of *Klebsiella pneumoniae* and *Escherichia coli* infecting humans in sub-Saharan Africa? Secondly, what is the prevalence of gut mucosal carriage of ESBL-E amongst humans in sSA, and what risk factors for carriage have been identified? To this end a search of PubMed and Scopus was carried out using the search terms given in the chapter appendix.

Inclusion criteria were any study that took place in sSA and allowed the calculation of a prevalence of ESBL-E in *K. pneumoniae* or *E. coli* amongst invasive human isolates, or prevalence of human gut mucosal carriage of ESBL-E. Studies were excluded if no ESBL-E confirmatory testing was performed using phenotypic (double disc or combination disc or E-test) or molecular (PCR) methods. Invasive isolates were defined to be any blood or CSF sample other usually sterile fluid, or urine or wound swabs with clinical suspicion of infection.

1.3.3.2 Statistical analysis

Data were extracted from the identified studies: prevalence of ESBL-E in *K. pneumoniae* or *E. coli* amongst invasive human isolates, or prevalence of human gut mucosal carriage of ESBL-E. Proportions were plotted in forest plots with exact binomial confidence intervals, and stratified by location of isolation in the case of carriage isolates (community, outpatient, on hospital admission, or inpatient). Summary estimates were calculated using random-effect meta analysis with generalised linear mixed models (GLMMs) assuming binomially distributed prevalences and normally distributed random effects - the normal-binomial model - using the R packages *meta*[65] v4.9.5 and *lme4* v1.1.21[182]. Heterogeneity was explored by meta-regression, particularly regressing proportion of ESBL producing *E. coli* and *K. pnemoniae* against year of isolation. This was achieved by adding year as a fixed effect covariate and assessing explanatory power of a model including the parameter to one without using likelihood ratio testing and considering $p < 0.05$ to be statistically significantly better fit. Predicted population prevalences were generated from the fitted models and plotted with 95% confidence intervals generated from 1000 bootstrap re-samples . Where available, data on the identified ESBL enzymes were also extracted and plotted as simple proportions.

1.3.3.3 Results

On 8th December 2018 this search identified 2975 unique studies; after abstract review 192 underwent full-text review, resulting in the inclusion of 86 studies, 54[183–235] providing data on invasive infection and 32 [236–268] on carriage. Details of these studies are given below and in . Of the 86 studies identified by the systematic literature review, 54 studies provided data on invasive ESBL-E and 32 provided data on human carriage is sSA. These are considered in turn below.

1.3.3.4 Invasive ESBL-E infection

Table 1.11 in the chapter appendix shows the 54 included studies in this analysis, which provide data on 6067 *E. coli* and 2974 *K. pneumoniae* isolates. All studies were cross sectional in design. Of the 54, 18/54 were laboratory based (i.e. a survey of all samples received in the laboratory); 17/54 were truly invasive in that they included predominantly blood culture; a combination of urine, CSF, and wound swabs were included in the remaining studies. 36/54 studies provided data on adults and children; 6/54 on adults only; and 12/54 on children only. The majority of studies (42/54) include both community and nosocomial acquired infection. Of the remainder, 3/54 provided data on nosocomial infection only. Figure 1.3A shows a map

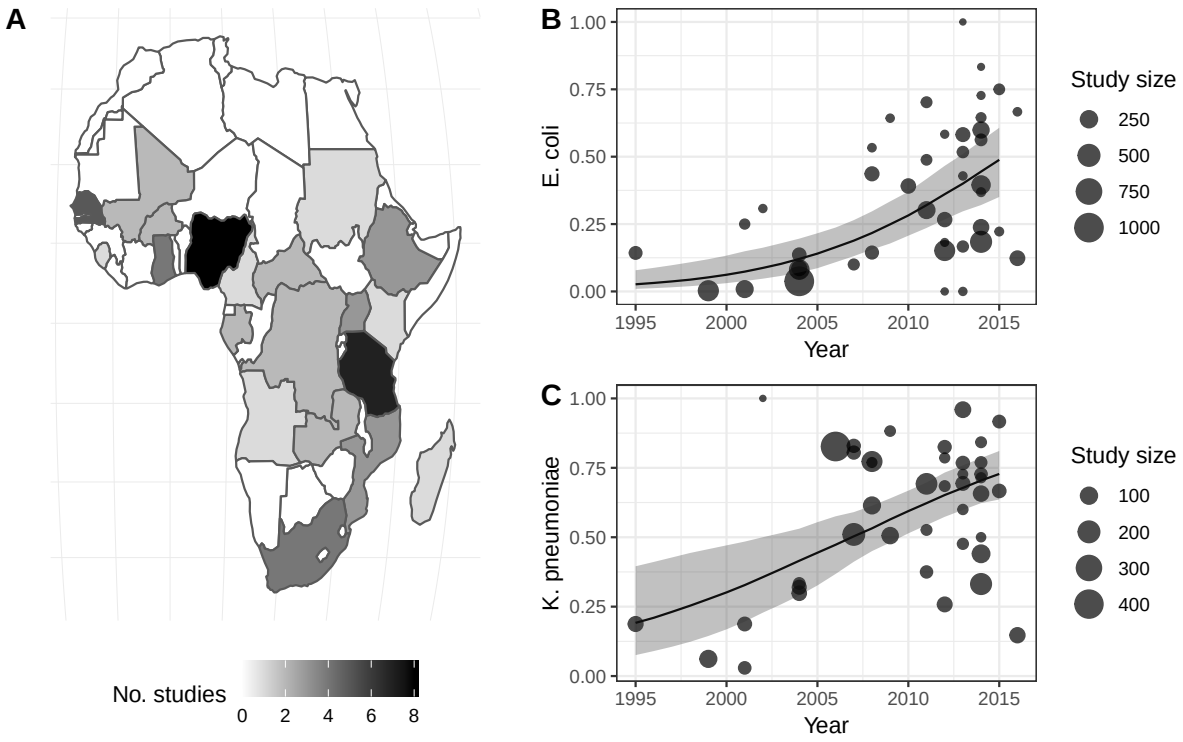

Figure 1.3: Invasive ESBL-E in sSA. A: Available studies by country. B and C: meta regression of proportion of invasive *E. coli* and *K. pneumoniae* respectively as a function of time. In both cases time is statistically significantly associated with proportion of ESBL ($p < 0.001$ on likelihood ratio testing of nested models). 95% CI generated from 1000 bootstrap replicates.

of available data by country; data are available from across the continent though Nigeria (8 studies) and Tanzania (7 studies) are over represented and many countries provide no data.

The proportion of ESBL producers amongst invasive E. coli and K. pneumoniae in sSA is heterogeneous but many studies show extremely an extremely high prevalence (Figures 1.3B and 1.3C), comparable to that seen in the Indian subcontinent and other high-prevalence areas and highlighting the scale of the public health problem posed by ESBL-E in sSA. Meta regression shows clear temporal trends of an increase over time: addition of time as a fixed-effect covariate to the random effects model gives improved fit on likelihood ratio testing of nested models ($p < 0.001$ for both *E. coli* and *K. pneumoniae*). Though data are sparse pre-2000, those data that are available suggest that ESBL producing *E. coli* and *K. pneumoniae* were identified in West Africa even in the 1990s: a retrospective laboratory based study in Yaounde, Cameroon on isolates from a variety of clinical samples from 1995-1998 found that 13/91 *E coli.* and 12/64 *K. pneumoniae* were ESBL producers, with the bla_{SHV-12} gene predominant[227]; in Dakar, Senegal, 6/97 *K. pneumoniae* isolates from community acquired urinary tract infections in 1999-2000 were found to be ESBL producers[230].

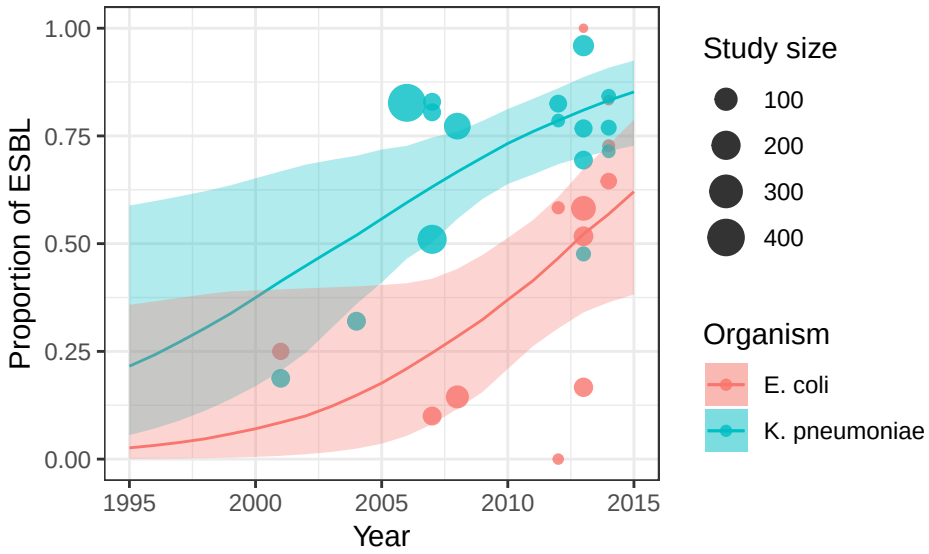

Figure 1.4: Meta regression of proportion of ESBL producing *E. coli* and *K. pneumoniae* amongst invasive isolate in sSA from studies carrying out blood culture, as a function of time. Includes 1242 *K. pneumoniae* and 489 *E. coli* isolates. 95% CI generated from 1000 bootstrap replicates from fitted models. In both cases time is statistically significantly associated with proportion of ESBL ($p < 0.001$ on likelihood ratio testing of nested models).

Some of the heterogeneity in prevalence does however seem to be explained by sample type; a clearer picture appears when the analysis is restricted to the 16 studies including predominantly blood culture (Figure 1.4)[187,189–194,196,199,200,202,204,209,212,226,235]. In this analysis it seems clear that the worldwide epidemiology of ESBL-E was mirrored in sSA; ESBL initially spread amongst invasive K. pneumoniae post 2000 (particularly post 2005) before becoming established in E. coli after 2010. In 2014, the latest available data, the pooled population prevalence of ESBL from binomal-normal random effects meta analysis was 61% [95% CI 40-80%] amongst *E. coli* and 86% [95% CI 73-92%] amongst *K. pneumoniae* bloodstream infection isolates, suggesting endemicity of ESBL amongst these pathogens in sSA, and comparable to the highest prevalence areas in the world.

ESBL genes were characterised in 10 studies by whole genome sequencing[235] (n=1) or by PCR[188,191,192,194,201,209,210,226,229] (n=9) for 821 *E. coli* and 791 *K. pneumoniae* isolates (Figure 1.5). bla_{CTX-M} were the most commonly occurring ESBL genes, and the majority of these were $bla_{CTX-M-15}$ in both organisms. bla_{OXA}, bla_{TEM} and bla_{SHV} genes were also commonly found but were often not further characterised, presenting some problems of interpretation, as these enzymes can be narrow or broad-spectrum beta-lactamases. Certainly, bla_{SHV-1} and bla_{TEM-1} encode narrow spectrum beta lactamase enzymes, which were commonly identified in these studies, though only a handful of isolates had characterisation of bla_{SHV} genes beyond identification of the bla_{SHV} group. All the identified bla_{OXA} genes

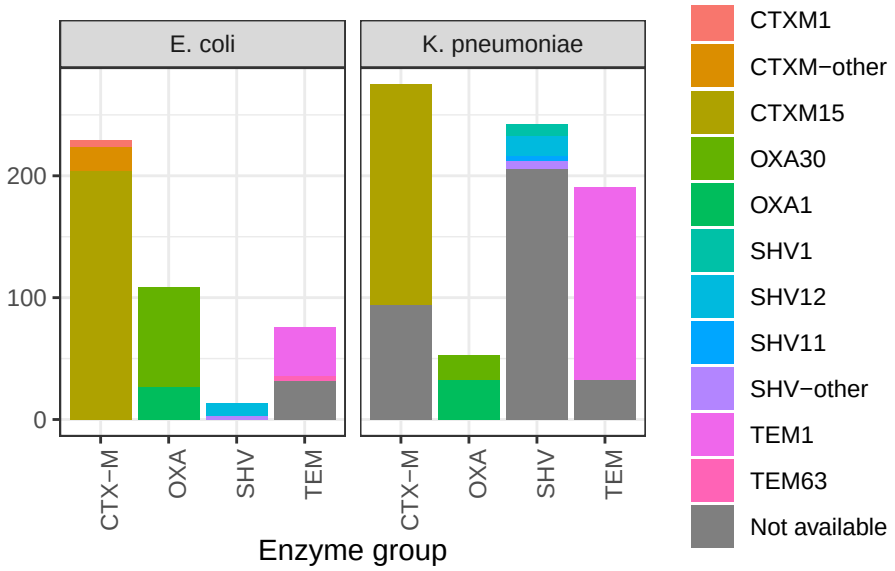

Figure 1.5: Disribution of beta-lactamase enzymes in invasive ESBL producing *E. coli* (n= 821) and *K. pneumoniae* (n = 791) from 10 studies

were narrow spectrum beta lactamases (bla_{OXA-1}). These data suggest that the genomic landscape of invasive ESBL-E in sSA is dominated by bla_{CTX-M}, and $bla_{CTX-M-15}$ in particular, mirroring that seen worldwide.

Though no data were identified from Malawi that fulfilled the inclusion criteria of the systematic review, there are three studies that suggest the epidemiology of invasive ESBL-E in Malawi is similar to that described above. A study from Blantyre in 2004-2005 found that ESBL-E were unusual in blood stream infection (BSI) isolates: of 1191 Enterobacteriaeciae BSI, only 8 unique isolates showed an ESBL phenotype (*K. pneumoniae* 4/8, *K. oxytoca*, 1/8, *Enterobacter cloacae* 2/8 and *E. coli* 1/8) though no denominators are provided to allow calculation of prevalence. In terms of ESBL enzymes, CTX-M-15 (n = 1) was described, though in the minority: SHV-11 (n = 1), SHV-12 (n = 3), SHV-27 (n = 1) and TEM-63 (n = 2) were the other enzymes identified[269]. Longitudinal blood culture surveillance in Blantyre suggests that after 2005 – which coincided with the introduction of ceftriaxone in government hospitals – the prevalence of ceftriaxone resistance rapidly increased, to 90.5% in K. pneumoniae and 30.3% in E. coli BSI isolates by 2016[11], though this study did not carry out confirmatory ESBL testing. Finally, two retrospective whole-genome sequencing study which selected 94 diverse (largely invasive) clinical *E. coli* and 72 *K. pneumoniae* isolates from Blantyre from 1996-2014 found that 21/94 *E. coli* isolates carried an ESBL gene, with CTX-M predominating (20/21)[270] and 31/60 *K. pneumoniae* had an ESBL phenotype again with $bla_{CTX-M-15}$predominant (39% [28/72] of identified ESBL genes)[271].

1.3.3.5 Gut mucosal carriage of ESBL-E in sub-Saharan Africa

Table 1.12 in the chapter appendix shows the 32 identified studies that provide data on gut mucosal carriage in different populations in sSA. The populations recruited to the studies are heterogeneous but include community members, hospitalised patients, outpatients, orphanage residents, hospital workers and food handlers in schools. Adults and children are included. Data on 10,232 individuals from 19 countries are available in total, and are plotted in Figure 1.6. The earliest samples were collected from staff and children in a Malian orphanage in 2003, when 49/68 participants were found to be colonised with ESBL-E[262]. There is significant heterogeneity in prevalence, some of which is explained by the study population (Figure **??**); inpatients tend to have a higher ESBL-E carriage prevalence than community members. Outpatients have similar carriage prevalence to community members but inpatients even on hospital admission seem to have a higher carriage prevalence than community members.

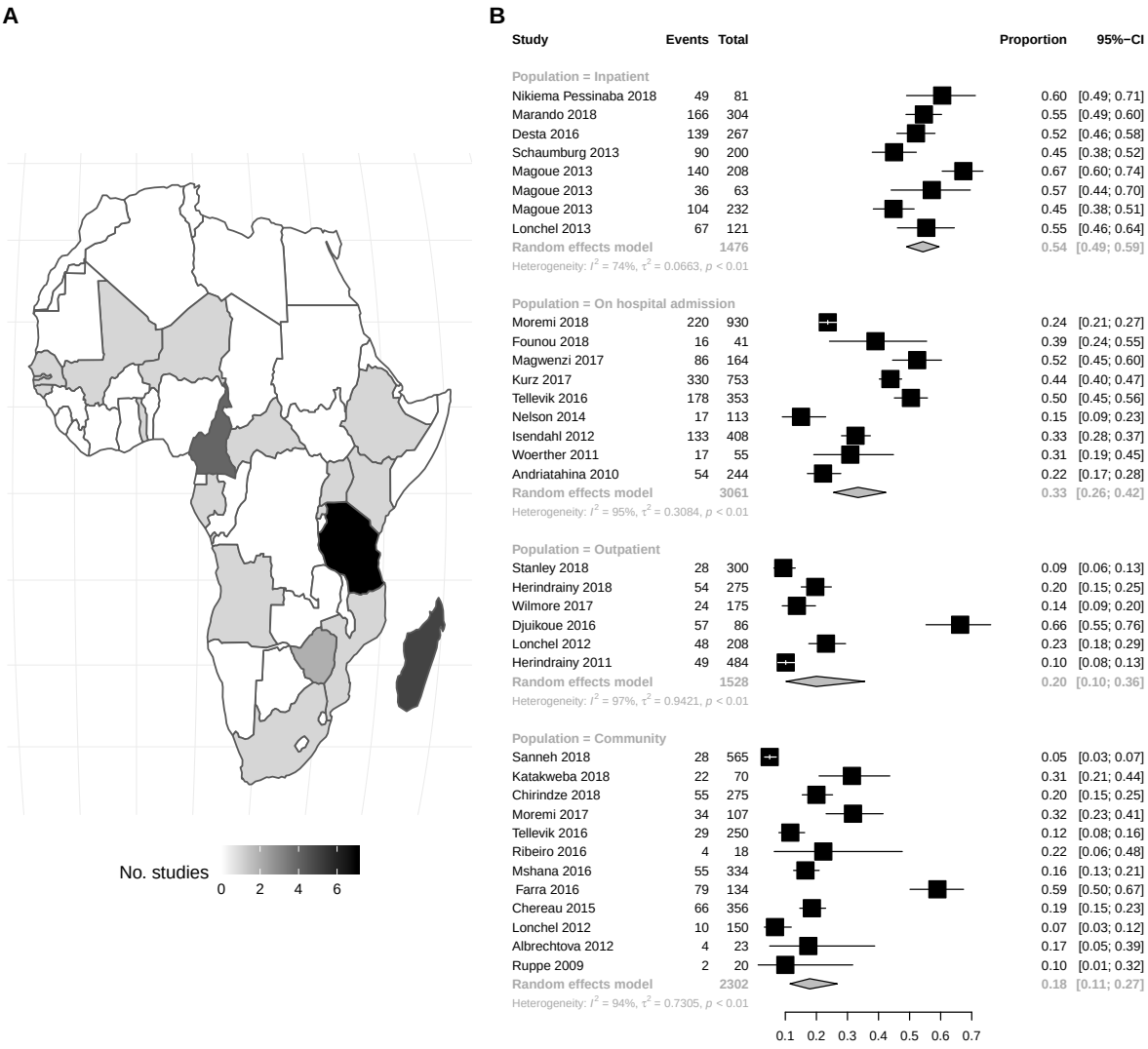

Figure 1.6: ESBL-E gut mucosal carriage in sSA. A: included studies by country; B: forest plot of ESBL-E carriage prevalence stratified by population. Pooled random effect summary estimates shown.

Significant heterogeneity in prevalence persists across all groups meaning that summary estimates should be interpreted with caution; community carriage if ESBL-E ranges from 5% in adults in The Gambia in 2015[266] to 59% in children in the Central African Republic in 2013[241], but a summary estimate from a random effect meta analysis is that 18% (95% CI 12-28%) of community members in sSA are colonised with ESBL-E, significantly higher than the prevalence in high-income settings.

Hospitalisation is clearly a driver of ESBL-E colonisation in the included studies - hospitalised cohorts have persistently higher prevalence of ESBL-E carriage – and prior antimicrobial exposure is consistently identified as a risk factor for carriage[243,250,266]. Consistent with a

putative faecal-oral transmission route, boiling water and using a borehole as a source of water were identified as protective factors in studies in Rwanda[265] and Togo[268] respectively. Data to elucidate the role of within-household transmission are sparse, though one study in Rwanda found that a colonised family member was independently associated with ESBL-E carriage on admission to hospital[268]. Lower socioeconomic status was found to both be protective against ESBL-E colonisation in the Central African Republic[241] and be associated with ESBL-E colonisation in Madagascar[257]; this relationship is likely to be complex and mediated by, for example, local availability and cost of antimicrobials. The role of HIV is not clear: in children in Dar-es-salaam, Tanzania, ESBL-E carriage was much more common amongst HIV infected children[246], and in Harare Zimbabwe, receipt of ART for less than a year was associated with carriage[240]. This relationship is very open to confounding and many studies have not found an association between ESBL-E carriage and HIV infection[243,250,256,264,266,268].

Data on ESBL enzymes present in carriage isolates are available for 996 E. coli and 607 K. pneumoniae from 8 studies (Figure **??**), showing a similar picture to invasive isolates; the landscape is dominated by CTX-M-15. One study used whole-genome sequencing [263], the remainder used a variety of PCR techniques[239,251–253,256,258,264].

Only 4 studies are longitudinal cohorts which could provide insight into temporal trends and determinants of carriage[258,260,264,268]; all of these studies were health facility based and ascertained ESBL status on admission and discharge. Significant increases in ESBL-E carriage were seen in all studies: from 50 to 65% in Rwanda; from 30 to 95% in Niger; from 21.2 to 57% in Madagascar; and from 23% to 36% in Tanzania. No studies followed patients into the community, thus carriage duration of ESBL-E in sSA remains unknown and no interventional studies identified aiming to reduce ESBL-E carriage were identified.

1.4 Conclusions

The aetiology of sepsis in sSA is poorly defined, hence optimal antimicrobial strategies are unknown; disseminated TB is likely to play a significant role, but data to guide tuberculosis therapy strategies in the critically unwell are lacking. The role of bacterial zoonoses, arboviruses and HIV opportunistic infections are not well defined, but may be significant. Diagnostic uncertainty and paucity of microbiologic support across sSA may be creating a permissive environment for the widespread broad spectrum antimicrobial use, often third-generation cephalosporins. It is likely that dose, duration and indication are frequently inappropriate, and thus could contribute both to increased mortality and to spread of ESBL-E.

Certainly, ESBL-E are endemic in sSA and are a problem of serious public health concern; sSA has rates of ESBL-E in invasive disease that are comparable to the highest in the world

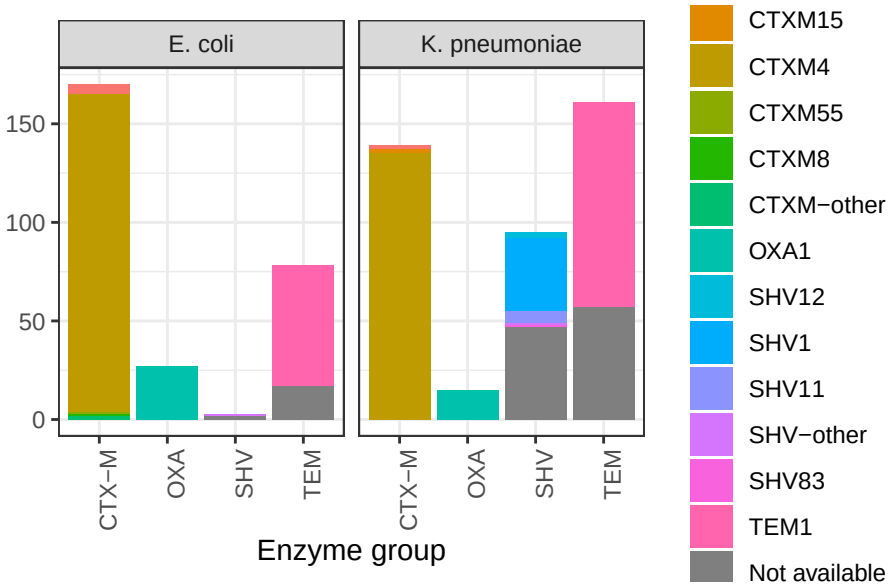

Figure 1.7: Distribution of beta-lactamase genes in carriage ESBL producing *E. coli* (n= 996) and *K. pneumoniae* (n = 607) from 9 studies.

and ESBL-E gut mucosal carriage in healthy populations across the continent is common. Whilst it is clear that health care facilities are strongly associated with ESBL-E acquisition, a deeper understanding of the determinants and sources of acquisition, and carriage duration is lacking. In order to understand the role of health facilities in driving the ESBL-E pandemic, a high quality longitudinal ESBL-E carriage data from both healthy and sick (health facility exposed) populations are required.

I propose that optimising the management of severe febrile illness can tackle two problems: reduce over prescription of broad spectrum antimicrobials and improve outcomes in critically unwell patients. This may seem counter-intuitive: why target the very unwell with an antimicrobial stewardship intervention? However I hypothesise that the "step-up" way in which we approach antimicrobial therapy in the immunosuppressed in resource limited settings is flawed, is driving the twin problems of poor sepsis outcomes and AMR, and may represent a low hanging fruit to tackle both problems. In a setting where so much management is empiric, current management begins with broad spectrum antibacterials and adds in further therapies - TB therapy, PCP therapy, or antifungals - based on non-response. This is the management that is codified in the WHO guidance for treating critically unwell TB suspects, but I suggest that it results in prolonged antibacterial exposure and delay in definitive treatment. We can imagine an alternate therapy, whereby we start broad, and rapidly narrow the spectrum of therapy based on the results of investigations. But such a strategy requires data; what are the causes of sepsis in sSA that we should target? How should we rationalise therapy? What are the determinants of AMR acquisition in sepsis survivors and how can we mitigate against

acquisition? It is the aim of this thesis to provide data to inform novel antimicrobial strategies for sepsis in Malawi and similar high-HIV high-TB prevalence settings throughout sSA.

1.4.1 Specific aims

The specific aims of this thesis are: 1. To describe the presentation, aetiology, outcome, and determinants of mortality from sepsis in adults presenting to Queen Elizabeth Central Hospital, Blantyre Malawi; 2. To describe the acquisition and carriage of ESBL-E in sepsis survivors, with an analysis of determinants of carriage.

1.5 Thesis overview

This thesis is based around a clinical study of sepsis in Blantyre, Malawi, which is described in Chapter 2. Chapter 3 presents data on the clinical presentation, aetiology and outcomes of sepsis in Blantyre, Malawi, with extended exploratory modelling of outcome in Chapter 4. Chapter 5 follows sepsis survivors out of the hospital on longitudinal stool sampling data to quantify ESBL-E carriage. Antibiotic-unexposed hospital controls and community members provide comparator cohorts to the antibacterial-exposed sepsis cohorts. To track bacteria and AMR-containing mobile genetic elements within study participants, I have used whole-genome sequencing of cultured isolates (WGS). Chapter 6 presents an overview of the genomic landscape of ESBL _E. coli_in Blantyre, whilst Chapter 7 outlines my attempts to use whole genome sequencing as a high-resolution typing tool to track AMR. Chapter 8 develops and fits longitudinal Markov models to understand the determinants of ESBL-E carriage in study participants, and brings in the genomic typing from the previous chapter. Finally, chapter 8 provides conclusions, and suggestions of further work.

1.6 Appendix

1.6.1 Search terms for sepsis literature review

Sepsis *and* ((Angola *or* Benin *or* Botswana *or* Burkina Faso *or* Burundi *or* Cameroon *or* Cape Verde *or* Central African Republic *or* Chad *or* Comoros *or* Republic of the Congo *or* Congo Brazzaville *or* Democratic republic of the Congo *or* Cote d'Ivoire *or* Djibouti *or* Equatorial Guinea *or* Eritrea *or* Ethiopia *or* Gabon *or* The Gambia *or* Ghana *or* Guinea *or* Guinea-Bissau *or* Kenya *or* Lesotho *or* Liberia *or* Madagascar *or* Malawi *or* Mali *or* Mauritania *or* Mauritius *or* Mozambique *or* Namibia *or* Niger *or* Nigeria *or* Reunion *or* Rwanda *or* Sao Tome and Principe *or* Senegal *or* Seychelles *or* Sierra Leone *or* Somalia *or* South Africa *or* Sudan *or* Swaziland *or* Eswatini *or* Tanzania *or* Togo *or* Uganda *or* Western Sahara *or* Zambia *or* Zimbabwe) *or* Africa).

1.6.2 Search terms for ESBL literature review

(((ESBL) **or** Extended-spectrum beta-lactamase)) *and* (((Angola *or* Benin *or* Botswana *or* Burkina Faso *or* Burundi *or* Cameroon *or* Cape Verde *or* Central African Republic *or* Chad *or* Comoros *or* Republic of the Congo *or* Congo Brazzaville *or* Democratic republic of the Congo *or* Cote d'Ivoire *or* Djibouti *or* Equatorial Guinea *or* Eritrea *or* Ethiopia *or* Gabon *or* The Gambia *or* Ghana *or* Guinea *or* Guinea-Bissau *or* Kenya *or* Lesotho *or* Liberia *or* Madagascar *or* Malawi *or* Mali *or* Mauritania *or* Mauritius *or* Mozambique *or* Namibia *or* Niger *or* Nigeria *or* Reunion *or* Rwanda *or* Sao Tome and Principe *or* Senegal *or* Seychelles *or* Sierra Leone *or* Somalia *or* South Africa *or* Sudan *or* Swaziland *or* Eswatini *or* Tanzania *or* Togo *or* Uganda *or* Western Sahara *or* Zambia *or* Zimbabwe) *or* Africa)).

Table 1.8: Sepsis diagnostic criteria

Definition	Diagnosis	Criteria
Sepsis-1 (1991)	SIRS	Two or more of: Temperature > 38°C or < 36°C, Heart rate > 90 /min, Respiratory rate > 20 /min or PaCO2 < 32mmHg (4.3 kPa), White blood cell count > 12 x 10-9 /Lor < 4 x 10-9 /L or > 10% immature forms
	Sepsis	SIRS plus proven or suspected infection
	Severe Sepsis	Sepsis plus acute organ dysfunction
	Septic shock	Sepsis with persistent hypotension after fluid resuscitation
Sepsis-2 (2001)	Sepsis	Infection documented or suspected and some of the following General variables: temperature > 38°C or < 36°C, heart rate > 90 min-1 or > SD above normal for age, tachypnoea, altered mental status, significant oedema or positive fluid balance (> 20ml/kg over 24hrs), hyperglycaemia > 7.7mmol /L Inflammatory variables: white blood cell count > 12 x 10-9 /L or < 4 x 10-9 /L or > 10% immature forms, plasma C-reactive protein > SD above normal, plasma procalcitonin > 2 SD above normal Haemodynamic variables: arterial hypotension (SBP < 90 mmHg or MAP < 70 mmHg or SBP decrease > 40mmHg in adults or 2SD below normal range, SvO2 > 70%, Cardiac index > 3.5
	Severe sepsis	Sepsis plus organ dysfunction Organ dysfunction variables: arterial hypoxaemia (PaO2 / FiO2) < 300, acute oliguria (urine output < 0.5 ml kg-1 hr -1 for at least 2 hours), creatinine increase > 0.5mg/ dL, coagulation abnormalities (INR > 1.5 or aPTT > 60s), ileus, thrombocytopenia (platelet count < 100,000 /mL, hyperbilirubinaemia (plasma bilirubin > 4mg /dL or 70 mmol /L
	Septic shock	Sepsis plus hypotension SBP < 90mmHg or MAP < 60mmHg or reduction in SBP of 40mmHg from baseline despite adequate volume resuscitation
Sepsis-3 (2016)	Sepsis	Infection plus life threatening organ dysfunction defined by an acute change in SOFA score of 2 or more
	Septic shock	Persisting hypotension requiring vasopressors to maintain MAP 65mmHg AND serum lactate below 2mmol /L

Note:
SIRS = Systemic Inflammatory Response Syndrome, SD = Standard deviation, SBP = Systolic blood pressure, MAP = Mean arterial blood pressure

Table 1.9: Sequential organ failure assessment (SOFA) score

	Score				
System	0	1	2	3	4
Respiratory					
Pao2 / FiO2 mmHg (kPa)	400 (53.3)	< 400 (53.3)	< 300 (40)	< 200 (26.7) with respiratory support	< 100 (13.3) with respiratory support
Coagulation					
Platelets x100,000/ mL	150	< 150	< 100	< 50	< 20
Liver					
Bilirubin mg /dL (mmol/ L)	<1.2 (20)	1.2-1.9 (20 – 32)	2.0 – 5.9 (33-101)	6.0 – 11.9 (102 – 204)	> 12.0 (204)
Cardiovascular					
Cardiovascular	MAP > 70mmHg	MAP < 70mmHg	Dopamine < 5 or dobutamine any dose	Dopamine 5.1 – 15 or epinephrine < 0.1 or norepinephrine < 0.1	Dopamine > 15 or epinephrine > 0.1 or norepinephrine > 0.1
CNS					
Glasgow coma scale	15	13-14	10-12	7-9	< 6
Renal					
Creatinine mg/dL (mmol /L)	< 1.2 (110)	1.2 – 1.9 (110 -170)	2.0 – 3.4 (171 – 299)	3.5 – 4.9 (300 – 440)	> 5.9 (440)
Urine output (ml /day)				< 500	< 200

Note:
PaO2 = Arterial partial pressure of oxygen, FiO2 = Inspired fraction of oxygen, MAP = mean arterial blood pressure, CNS = Central nervous system. All doses of inotropes are micrograms/kg/min

Table 1.10: Selected causes of fever in sSA since 2013

Study	Year	Country	Setting	Patient Population	Test used	Case definition	Confirmed acute disease
Leptospirosis							
Zida 2018	2014-15	Burkina Faso	Central reference lab	Febrile Jaundice adults and children	In house IgM followed by MAT and PCR (acute only, > 1:400)	MAT > 1:400	27/781 (3.5%)
Guillebaud 2018	2014-2015	Madagascar	21 health-care centres	Febrile adults and children	PCR array	Positive PCR	1/682 (0.2%)
Maze 2018	2012-2014	Tanzania	2 Referral Hospitals	Febrile adults and children	MAT (acute + conv)	MAT > 1:800 or fourfold rise	24/1239 (1.9%)
Gadia 2017		Central African Republic	Central reference lab	Febrile Jaundice adults and children	IgM ELISA (acute only)	Any IgM positive	0/198 (0%)
Hagen 2017	2011-2013	Madagascar	District Hospital	Adults and children FUO	PCR	Positive PCR	0/1009 (0%)
Biscornet 2017	2014-2015	Seychelles	Reference leptospirosis clinic	13 or above FUO, referred to central leptospirosis clinic	In house IgM followed by MAT and PCR (acute + conv)	MAT > 1:400 or fourfold rise	51/225 (23%)
Dreyfus 2017	2014	Uganda	2 Health centres	Any adult heath centre attendee	MAT (acute only)	MAT > 1:800	7/359 (1.9%)
Hercik 2017	2014-2015	Tanzania	District hospital	Febrile adults and children	Taqman PCR array	Positive PCR	22/842 (2.6%)

Table 1.10: Selected causes of fever in sSA since 2013 *(continued)*

Study	Year	Country	Setting	Patient Population	Test used	Case definition	Confirmed acute disease
Chipwaza 2015	2014	Tanzania	District hospital	Outpatient febrile children	IgM IgG ELISA then MAT (acute only)	MAT > 1:160	26/200 (13%)
Q-fever							
Amoako 2018	2016-17	Ghana	2 distrct hospitals	Febrile children	Taqman PCR array	Positive PCR	1/166 (0.6%)
Hercik 2017	2014-2015	Tanzania	District hospital	Febrile adults and children	Taqman PCR array	Positive PCR	2/842 (0.2%)
Boone 2017	2011-13	Madagascar	Two public health care facilities	Febrile adults and children	PCR	Positive PCR	0/1005
Njeru 2016	2014-15	Kenya	Two district hospitals	Febrile adults and children	Phase I/II IgG ELISA and IFA; PCR on subset (acute only)	Phase II IgG IFA titre > 1:128	163/1067 (15%), 10/448 (2.2%) PCR positive
Mourembou 2016	2013-14	Gabon	Four health centres	Febrile children	PCR	Positive PCR	0/410 (0%)
Maina 2016	2011-12	Kenya	District Hospital	Febrile children	IgM/IgG ELISA phase I and II (acute and conv)	Phase II IgG seroconversion	25/370 (8.9%)
Angelaksis 2014	2010-12	Senegal, Mali, Gabon	Six health centres	Febrile adults and children	PCR	Positive PCR	6/1388 (0.4%)
Brucellosis							

Table 1.10: Selected causes of fever in sSA since 2013 *(continued)*

Study	Year	Country	Setting	Patient Population	Test used	Case definition	Confirmed acute disease
Cash-Goldwasser 2018	2012-14	Tanzania	Two referral hospitals	Febrile adults and children	MAT and blood culture (acute + conv)	Fourfold rise in MAT	39/562 (6.9%)
Gafiritia 2017	2014	Rwanda	District hospital	Adults, fever	Rose Bengal test	Positive test	10/198 (6.1%)
Boone 2017	2011-13	Madagascar	Two public health care facilities	Febrile adults and children	PCR	Positive PCR	15/1005 (1.5%)
De Glanville 2017	2012	Kenya	Referral hospital and private clinic	Febrile adults and children	Rose Bengal test	Positive test	8/825 (9.7%)
Njeru 2016	2014-15	Kenya	Two district hospitals	Febrile adults and children	Rose bengal test, IgG/IgM ELISA, PCR (acute only)	Positive ELISA or PCR	146/1067 (13.7%)
Chipwaza 2015	2014	Tanzania	District hospital	Outpatient febrile children	IgM and IgG and tube agglutination (acute only)	Positive IgM	26/370 (7.0%)
Feleke 2015	2011	Ethiopia	Health centre	Febrile adults and children	Brucella antigen test	Positive test	3/280 (1%)
Rickettsioses							

Table 1.10: Selected causes of fever in sSA since 2013 *(continued)*

Study	Year	Country	Setting	Patient Population	Test used	Case definition	Confirmed acute disease
Amoako 2018	2016-17	Ghana	2 distrct hospitals	Febrile children	Taqman PCR array	Positive PCR	5/166 (3.0%) RS
Hercik 2017	2014-2015	Tanzania	District hospital	Febrile adults and children	Taqman PCR array	Positive PCR	2/842 (0.2%) RF
Sothmann 2017	2012	Ghana	Referral hospital	Febrile Children	PCR	Positive PCR	6/431 (1.4%) RF
Maina 2016	2011-12	Kenya	District Hospital	Febrile children	IgG ELISA (acute and conv)	Fourfold rise in IgG titre	63/364 (22.4%) SFG 3/364 (1.1%) TG, 10/364 (3.6%) STG
Elfving 2016	2011	Zanzibar	District hospital	Febrile children with no diagnosis	PCR	Positive PCR	0/83 RS
Mourembou 2015	2013-14	Gabon	4 heath centres	Febrile children	PCR	Positive PCR	42/410 (10.2%) RF
Dengue							
Amoako 2018	2016-17	Ghana	2 district hospitals	Febrile children	Taqman PCR array	Positive PCR	2/166 (1.2%)
Guillebaud 2018	2014-2015	Madagascar	21 health-care centres	Febrile adults and children	PCR macroarray	Positive PCR	0/682 (0%)
Kayiwa 2018	2014-2017	Uganda	District hospital	Febrile adults and children	PCR	Positive PCR	1/384 (0.26%)
Makiala-Mandanda 2018	2003-2012	Democratic Republic of Congo	Central lab	Febrile Jaundice, yellow fever IgM negative	PCR	Positive PCR	16/453 (3.5%)

Table 1.10: Selected causes of fever in sSA since 2013 *(continued)*

Study	Year	Country	Setting	Patient Population	Test used	Case definition	Confirmed acute disease
Muianga 2018	2014	Mozambique	Not clear	Febrile adults and children	IgG, IgM and PCR (acute only)	Positive PCR	37/99 by PCR (37.4%)
Mugabe 2018	2016	Mozambique	Five health centres	Febrile adults and children	IgM, IgG, PCR (acute only)	Positive PCR	PCR 0/163
Hercik 2018	2014-2015	Tanzania	District hospital	Febrile adults and children	Taqman PCR array	Positive PCR	1/191 (0.5%)
Gadia 2017		Central African Republic	Central reference lab	Febrile Jaundice adults and children	IgM (Acute only)	Positive IgM	0/198 (0%)
Vu 2017	2014-2015	Kenya	Two health centres	Febrile children	PCR	Positive PCR	82/1104 (7.4%)
Waggoner 2017	2014-2015	Kenya	Two health centres and two district hospitals	Children with fever	PCR	Positive PCR	0/385 (0%)
Kolawole 2017	2016	Nigeria	Two heath centres	Adults and children with fever	IgM, IgG, PCR (Acute only)	Positive PCR	11/176 (6.2%)
Nasir 2017	2016	Nigeria	Teaching hospital	Adults and children with fever	NS1 antigen	Positive antigen	15/171 (8.8%)
Ngoi 2016	2014-2015	Kenya	Five health clinics, one district hospital	Adults with fever, negative for acute HIV and malaria	PCR	Positive PCR	43/489 (8.8%)

Table 1.10: Selected causes of fever in sSA since 2013 *(continued)*

Study	Year	Country	Setting	Patient Population	Test used	Case definition	Confirmed acute disease
Onoja 2016	2014	Nigeria	One district hospital	Adults and children with fever	IgM (Acute only)	Positive IgM	64/274 (23.3%)
Kajeguka 2016	2013-2014	Tanzania	Three district hospitals	Probable Dengue (on clinical and IgM)	PCR	Positive PCR	0/381 (0%)
Elfving 2016	2011	Zanzibar	District hospital	Febrile children with no diagnosis	PCR	Positive PCR	0/83
Sow 2016	2009-2013	Senegal	Seven health-care facilities	Adults and children with fever	IgM, PCR (acute only)	Positive PCR	3/13,845 (0.02%)
Chipwaza 2014	2013	Tanzania	One district hospital	Children with fever	IgM, PCR (acute only)	Positive PCR	29/364 (8.0%)
Chikingunya							
Kayiwa 2018	2014-2017	Uganda	District hospital	Febrile adults and children	PCR	Positive PCR	19/384 (4.9%)
Makiala-Mandanda 2018	2003-2012	Democratic Republic of Congo	Central lab	Febrile Jaundice, yellow fever IgM negative	PCR	Positive PCR	2/453 (0.4%)
Muianga 2018	2014	Mozambique	Not clear	Febrile adults and children	IgG, IgM (acute only)	Positive IgM	8/114 by IgM (7%)
Antonio 2018	2015-16	Mozambique	Eight health centres	Undifferentiated fever	IgM, IgG (Acute only)	Positive IgM	6/392 (1.5%)
Mugabe 2018	2016	Mozambique	Five health centres	Febrile adults and children	IgM, IgG, PCR (Acute only)	Positive PCR	PCR 0/163, IgM 17/163 (10.4%)

Table 1.10: Selected causes of fever in sSA since 2013 *(continued)*

Study	Year	Country	Setting	Patient Population	Test used	Case definition	Confirmed acute disease
Sow 2017	2009-2010	Senegal	Fiver health centres and four schools	Febrile adults and children	IgM, IgG, PCR (Acute only)	Positive PCR	20/1049 (1.4%)
Gadia 2017		Central African Republic	Central reference lab	Febrile Jaundice adults and children	IgM (Acute only)	Positive IgM	0/198 (0%)
Olajiga 2017	2015-2016	Nigeria	Seven hospitals	Fever or joint pain or rash, over 10 years	IgM, IgG (acute only)	Positive IgM	66/172 (38.4) by IgM
Waggoner 2017	2014-2015	Kenya	Two health centres and two district hospitals	Children with fever	PCR	Positive PCR	32/385 (8.3%)
Ngoi 2016	2014-2015	Kenya	Five health clinics, one district hospital	Adults with fever, negative for acute HIV and malaria	PCR	Positive PCR	0/489 (0%)
Kajeguka 2016	2013-2014	Tanzania	Three district hospitals	Probable Chikungunya (on clinical and IgM)	PCR	Positive PCR	11/263 (4.2%)
Elfving 2016	2011	Zanzibar	District hospital	Febrile children with no diagnosis	PCR	Positive PCR	0/83
Sow 2016	2009-2013	Senegal	Seven health-care faciities	Adults and children with fever	IgM, PCR (acute only)	Positive PCR	13/13,845 (0.1%)
Chipwaza 2014	2013	Tanzania	One district hospital	Children with fever	IgM (acute only)	Positive IgM	17/364 (4.7%)
Zika							

Table 1.10: Selected causes of fever in sSA since 2013 *(continued)*

Study	Year	Country	Setting	Patient Population	Test used	Case definition	Confirmed acute disease
Kayiwa 2018	2014-2017	Uganda	District hospital	Febrile adults and children	PCR	Positive PCR	5/384 (1.3%)
Makiala-Mandanda 2018	2003-2012	Democratic Republic of Congo	Central lab	Febrile Jaundice, yellow fever IgM negative	PCR	Positive PCR	0/453 (0%)
Sow 2016	2009-2013	Senegal	Seven health-care faciities	Adults and children with fever	IgM, PCR (Acute only)	Positive PCR	9/13,845 (0.1%)

Note:
RS = Rickettsia spp., RF = R. felis, SFG/TG/STG = spotted fever/ typhus/scrub typhus group

Table 1.11: included studies providing an estimate of proportion of ESBL producers in invasive *E. coli* and *K. pneumoniae* isolates in sSA.

Year	First author	Country	Population	Sample	*E. coli*	*K. pneumoniae*
2018	Guiral	Mozambique	A C IP	Blood urine	13/151 (9%)	ND
2018	Karppinen	Angola	C IP OP	Wound swab	8/15 (53%)	10/13 (77%)
2018	Kpoda	Burkina Faso	A C IP OP	Various	117/296 (40%)	48/109 (44%)
2018	Onanuga	Nigeria	A OP	Urine	4/18 (22%)	30/45 (67%)
2018	Seni	Nigeria	A C IP OP	Various	41/60 (68%)	ND
2018	Zeynudin	Ethiopia	NA IP OP	Various	13/13 (100%)	30/31 (97%)
2017	Ampaire	Uganda	A C IP OP	Various	18/146 (12%)	10/68 (15%)
2017	Andrew	Uganda	A C IP OP	Various	33/44 (75%)	33/36 (92%)
2017	Archary	South Africa	C IP	Various	2/11 (18%)	13/19 (68%)
2017	Henson	Kenya	A C IP OP	Blood	ND	101/198 (51%)
2017	Ibrahim	Nigeria	A C IP OP	Urine wound swab	68/140 (49%)	76/108 (70%)
2017	Kassam	Tanzania	A C IP OP	Wound swab	6/14 (43%)	8/11 (73%)
2017	Legese	Ethiopia	C IP	Blood urine	5/6 (83%)	16/19 (84%)
2017	Manyahi	Tanzania	A C IP OP	Urine	15/110 (14%)	9/27 (33%)
2017	Sangare	Mali	A C IP	Blood	20/31 (65%)	20/26 (77%)
2017	Vasaikar	South Africa	A C IP OP	Various	ND	117/169 (69%)
2016	Abera	Ethiopia	A C IP OP	Blood urine	71/122 (58%)	34/49 (69%)
2016	Agyekum	Ghana	A C IP OP	Blood urine	30/58 (52%)	33/43 (77%)
2016	Breurec	Senegal	C IP	Blood CSF	ND	33/41 (80%)
2016	Buys	South Africa	C IP	Blood	ND	339/410 (83%)
2016	Eibach	Ghana	A C IP	Blood	5/50 (10%)	34/41 (83%)
2016	Kabwe	Zambia	C IP	Blood	5/5 (100%)	71/74 (96%)
2016	Leski	Sierra Leone	A C OP	Urine	0/13 (0%)	9/15 (60%)
2016	Mohammed	Nigeria	A C IP OP	Various	41/172 (24%)	59/178 (33%)
2016	Naas	Madagascar	C IP OP	Blood	0/7 (0%)	11/14 (79%)
2016	Ndir	Senegal	C IP	Blood	7/12 (58%)	33/40 (82%)
2016	Ouedraogo	Burkina Faso	A C IP OP	Various	121/202 (60%)	46/70 (66%)
2016	Sangare	Mali	A C IP	Blood	8/11 (73%)	10/14 (71%)
2016	Seni	Tanzania	A IP	Pertitoneal fluid	7/19 (37%)	5/10 (50%)
2015	Dramowski	South Africa	C IP OP	Blood	14/97 (14%)	119/154 (77%)

Table 1.11: included studies providing an estimate of proportion of ESBL producers in invasive *E. coli* and *K. pneumoniae* isolates in sSA. *(continued)*

Year	First author	Country	Population	Sample	*E. coli*	*K. pneumoniae*
2015	Irenge	Democratic Republic of Congo	A C IP OP	Blood	9/54 (17%)	10/21 (48%)
2015	Kateregga	Uganda	A C IP OP	Various	36/64 (56%)	24/33 (73%)
2015	Opintan	Ghana	A C IP OP	Various	81/440 (18%)	ND
2015	Pons	Mozambique	A C IP OP	Blood urine	ND	16/50 (32%)
2015	Rafa	Central african republic	A C IP	Wound swab	33/47 (70%)	10/19 (53%)
2014	Adeyankinnu	Nigeria	A C IP OP	Various	36/135 (27%)	16/62 (26%)
2014	Irenge	Democratic Republic of Congo	A C IP OP	Urine	57/376 (15%)	ND
2014	Scherbaum	Gabon	A IP	Various	5/14 (36%)	3/6 (50%)
2014	Yusuf	Nigeria	A IP OP	Various	47/278 (17%)	19/128 (15%)
2013	Alabi	Gabon	A C IP OP	Various	ND	43/85 (51%)
2013	Ibrahim	Sudan	A C IP OP	Various	70/232 (30%)	ND
2013	Obeng-Nkrumah	Ghana	A C IP OP	Various	55/126 (44%)	59/96 (61%)
2013	Raji	Nigeria	A C IP OP	Various	21/43 (49%)	12/32 (38%)
2013	van der Meeren	Mozambique	C IP	Urine	9/14 (64%)	15/17 (88%)
2011	Idowu	Nigeria	A IP	Wound swab	6/15 (40%)	ND
2010	Moyo	Tanzania	A C IP OP	Urine	54/138 (39%)	ND
2009	Bercion	Central African Republic	A C OP	Urine	29/357 (8%)	17/57 (30%)
2009	Mshana	Tanzania	A C IP OP	Various	31/127 (24%)	58/91 (64%)
2007	Sire	Senegal	A C IP OP	Urine	38/1010 (4%)	ND
2005	Blomberg	Tanzania	C IP OP	Blood	9/36 (25%)	9/48 (19%)
2005	Gangoue Pieboji	Cameroon	A C IP	Various	13/91 (14%)	12/64 (19%)
2005	Ndugulile	Tanzania	A IP	Various	4/13 (31%)	2/2 (100%)
2004	Dromigny	Senegal	A C OP	Urine	2/233 (1%)	1/34 (3%)
2002	Dromigny	Senegal	A C OP	Urine	1/386 (0%)	6/97 (6%)

Note:

A = Adults, C = children, IP = inpatients OP = outpatients, ND = not done.

Table 1.12: Included studies providing estimate of prevalance of ESBL-E gut mucosal colonisation in sSA.

Study	Year Pub.	Study Period	Country	Study Type	Inclusion	Age group	Median age	n
Ruppe	2009	NR	Senegal	Cross sec.	Children in village selected for remoteness	Children	6.9yr*	20
Tande	2009	2003	Mali	Cross sec.	Orphanage children	Children	NR	38
					Orphanage staff	Adults	NR	30
Andriatahina	2010	2008	Madagascar	Cohort	Inpatients	Children	38.3m	244
Herindrainy	2011	2009	Madagascar	Cross sec.	Health centre attendees	Adults	NR	306
					Health centre attendees	Children	NR	147
Woerther	2011	2007-08	Niger	Cohort	Children with SAM, inpatients	Children	16.3m*	55
Albrechtova	2012	2009	Kenya	Cross sec.	Community members	Adults	NR	23
Isendahl	2012	2010	Guinea-Bissau	Cross sec.	Children att. hospital w/ fever or tachycardia	Children	NR	408
Lonchel	2012	2009	Cameroon	Cross sec.	Students in the community	Adults	24.7yr*	150
					Outpatients	Adults	36.9yr*	208
Lonchel	2013	2009	Cameroon	Cross sec.	Inpatients	Adults	46.8yr*	121
Magoue	2013	2010	Cameroon	Cross sec.	Hospital workers and their families	Adults	NR	87
					Inpatients	Adults	NR	208
					Relatives and carers of inpatients	Adults	NR	63
					Outpatients	Adults	NR	232
Schaumburg	2013	2010-11	Gabon	Cross sec.	Hospital inpatients	Children	NR	200
Nelson	2014	2013	Tanzania	Cohort	Pregnant women and neonates, inpatient	Neonate	0d	126
						Adults	26.5yr*	113
Chereau	2015	2013-14	Madagascar	Cross sec.	Pregnant women in the community	Adults	26yr*	356
Desta	2016	2012	Ethiopia	Cross sec.	Inpatients	Adults	35yr	154
					Inpatients	Children	7yr	94
					Inpatients	Neonate	9d	19

Table 1.12: Included studies providing estimate of prevalance of ESBL-E gut mucosal colonisation in sSA. *(continued)*

Study	Year Pub.	Study Period	Country	Study Type	Inclusion	Age group	Median age	n
Djuikoue	2016	2011-12	Cameroon	Cross sec.	Outpatient women with susp. UTI	Adults	NR	86
Farra	2016	2013	CAR	Cross sec.	Healthy community controls from diarrhoea study	Children	10.5m	134
Kurz	2016	2014	Rwanda	Cohort	Inpatients and one main caregiver	both	29yr	753
Mshana	2016	2014	Tanzania	Cross sec.	Community members	both	10yr	334
Ribeiro	2016	2013	Angola	Cross sec.	Community members no antibiotics/hospital exposure last 3 m	Adults	NR	18
Tellevik	2016	2010-11	Tanzania	Cross sec.	<2yr attending health centre for vaccine	Children	NR	250
					Inpatients	Children	NR	353
Magwenzi	2017	2015	Zimbabwe	Cohort	Inpatient within 24hr of admission	Children	1.0yr	164
Moremi	2017	2015	Tanzania	Cross sec.	Street children	Children	14.2yr*	107
Wilmore	2017	2014-15	Zimbabwe	Cross sec.	Outpatient, HIV infected, stable on ART	Children	11yr	175
Chirindze	2018	2016	Mozambique	Cross sec.	Students in the community	Adults	NR	275
Founou	2018	2017	South Africa	Cohort	On hospital admission	Adults	NR	43
Herindrainy	2018	2015-16	Madagascar	Cross sec.	Pregnant women at delivery (home/facility)	Adults	26yr*	275
Katakweba	2018	2011-13	Tanzania	Cross sec.	Community members	Adults	NR	70
Marando	2018	2016	Tanzania	Cross sec.	Neonates with sepsis	Neonate	6d	304
Moremi	2018	2014-15	Tanzania	Cohort	On hospital admission	Adults	NR	930
Nikema	2018	2015-16	Togo	Cross sec.	<5yr with febrile gastroenteritis	Children	NR	81
Sanneh	2018	2015	The Gambia	Cross sec.	Food handlers in schools	Adults	37yr*	565

Table 1.12: Included studies providing estimate of prevalance of ESBL-E gut mucosal colonisation in sSA. *(continued)*

Study	Year Pub.	Study Period	Country	Study Type	Inclusion	Age group	Median age	n
Stanley	2018	2017	Uganda	Cross sec.	Participants who reared animals, attending health facility with a fever and/or diarrhoea but without malaria	both	21.7yr*	300

Note:
NR = Not reported, CAR = Central African Republic.
* Mean not median

1.7 References

Chapter 2

Methods

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Whole genome sequencing as a high-resolution typing tool to track longitudinal ESBL-E colonisation

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Chapter 9

Conclusions and further work

9.1 Introduction

In this thesis, I have presented the findings from a clinical study with two broad aims: first, to describe the presentation, aetiology, outcomes and determinents of outcomes in adults in Blantyre, Malawi; and second, to describe colonisation with ESBL-E as sepsis survivors pass from he hospital to the community, and an analysis to identify determinents of carriage. In Chapter one, I presented a conjecture that it is possible to improve outcomes for patients with sepsis whilst reducing over-reliance on broad spectrum antibacterials such as ceftriaxone, and hence minimise pressure for the development of antimicrobial resistance. In this chapter I review the findings of this thesis in the context of this conjecture, and suggest directions for future work.

9.2 Summary of findings

The clinical study of sepsis presented in Chapters 3 and 4 suggests that sepsis is Malawi is very different from sepsis in high-income low-HIV low-TB prevalence settings from where most studies of sepsis arise. Patients presenting with sepsis, as elsewhere in sSA, are young, and predominantly HIV-infected. They have been sick for a prolonged period of time - a median on 7 days - and, perhaps related to this, the identfied pathogens are those that may be less associated with fulminant disease than the Gram-negatives that cause sepsis in the hospitals of Europe, America and other high income settings: *Salmonella* Typhi bloodstream infection, disseminated tuberculosis, and malaria. The easily modifiable determinants of sepsis outcome that have receieved so much attention in high-income settings - rapid administration

of antibacterials and fluids - were not associated with survival in this cohort. This could be due to a true lack of effect, or to underpowering, but it highlights the need for data from sSA to guide sepsis protocols for sSA.

TB was the commonest cause of sepsis identified in the cohort - 34% of participants had at least one positive diagnostic test for TB - and that administration of antituberculous chemotherapy showed an association with survival (RR 1.25 for survival [95% CI 0.04-1.51] in propensity-score analysis). The role of TB therapy in the treatment of the critically unwell is unknown, and should be the focus of further work (see below). The 28-day mortality of sepsis in Blantyre was lower than expected at 18% (95% CI 13-23%), and lower than historical mortality estimates from across the continent described in Chapter 1. The reasons for this are speculative, but ART coverage was high compared to historical cohorts. Despite this, it seems likely that the presentation of sepsis in a number of participants in this study was a manifestation of ART failure. Few participants were switched to second-line therapy, and longer-term mortality (beyond 28 days) was signifiant in the HIV-infected with a near doubling of mortality from 19% (95% CI 13-26%) at 28 days to 36% (95% CI 28-45%) at 180 days.

In Chapter 5 I presented details of gut mucosal colonisation with ESBL-E in sepsis participants but also antibiotic-unexposed hospital inpatients and community members. Community carriage is common, with a baseline community carriage prevalence of 28% (95% CI 20-38%) and factors associated with baseline carriage strongly suggest that within-household and environmental transmission routes are important: houshold crowding, unprotected water source and sample collection during rainy season. Co-trimoxazole preventative therpay (CPT) was also associated with ESBL-E carriage at abseline, as was recent hospitalisation. In antibacterial-exposued inpatients there is a dramatic rise in ESBL-E colonisation prevalence, which is not seen in the antibacterial-unexposed. As expected, broad spectrum antibacterial exposure, largely ceftriaxone, was near-universal in the sepsis cohort with a median 5 (IQR 3-7) of exposure, but this is dwarfed in person-days of exposure by co-trimoxazole. This is perhaps unsurprising given that co-trimoxazole is mandated lifelong for all people loving with HIV in Malawi, but the magnitude of the difference is almost two orders of magnitude: 14,447 person days of exposure of co-trimoxazole verusus 997 for ceftriaxone.

Genomic analyses using whole genome sequencing of a subset of cultured isolates suggests that there is reasonably unrestricted mixing of *E. coli* strains at multiple spatial levels: between Malawi and the the rest of the world, and between the hospital and community. The exception to this is a putative recently arrived high-risk clone, ST410, which shows some healthcare association. The genomic landscape of ESBL in Blantyre is dominated by bla_{CTX-M}, reflecting the global situaion, and, worryingly, I describe (to my knowledge) the first identified carbapenamase in Malawi, a bla_{NDM-5} carried on a globally sucessful IncX

plasmid. Other idenitified AMR genes reflect the local antibiotic pressures: in particular, co-trimoxazole resistance determinents are near-ubiquitous. Using WGS as a high-resolution typing tool by clustering bacteria core genes and ESBL-containing contigs I show that it is the ESBL contig-bacteria combination that is conserved within patients, suggesting that the unit of transmission in this system is the bacteria, rather than transfer of MGE. I also demonstrate signifiant turnover of ESBL-E: beyond 35 days, two samples from within a patient are no more likley to contain the same ESBL contig-bacteria combination than due to chance alone.

Finally, I develop and fit two-state continuous-time Markov models in a Bayesian framework to understand the drivers of ESBL-E carriage in this cohort. I find that hospitalisation increases both ESBL-E acquisition and loss with a net effect of rapid ESBL-E acquisition following hospitalisation. Antibacterial exposure acts to prolong carriage by reducing the rate of loss, and I find support in the data for a prolonged post-antibiotic effect with a long half-life of 43 days (95% CI 15-98 days) that acts to prolong carriage long after antibacterial exposure has finished. Simulations show that there is syngery between hospitalisation and antibacterial exposure which produces the sharp rises in ESBL-E colonisation prevalence, but also that the post-antibiotic effect results in significantly prolonged carriage. In addition, the post-antibiotic effect means that short courses of antibacterials have, in terms of ESBL-E carriage, similar effects to prolonged courses, with significant implications for antimicrobial stewardship. Co-trimoxazole preventative therapy seems to be a significant driver of long-term carriage.

9.3 Future research priorities

References

1 Majno G. The Ancient Riddle of Formula (Sepsis). *Journal of Infectious Diseases* 1991;**163**:937–45. doi:10.1093/infdis/163.5.937

2 Singer M, Deutschman CS, Seymour CW *et al.* The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016;**315**:801. doi:10.1001/jama.2016.0287

3 Poll T van der, Veerdonk FL van de, Scicluna BP *et al.* The immunopathology of sepsis and potential therapeutic targets. *Nature reviews Immunology* 2017;**17**:407–20. doi:10.1038/nri.2017.36

4 Fleischmann C, Scherag A, Adhikari NKJ *et al.* Assessment of Global Incidence and Mortality of Hospital-treated Sepsis. Current Estimates and Limitations. *American Journal of Respiratory and Critical Care Medicine* 2016;**193**:259–72. doi:10.1164/rccm.201504-0781OC

5 Kaukonen K-M, Bailey M, Suzuki S *et al.* Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000-2012. *JAMA* 2014;**311**:1308–16. doi:10.1001/jama.2014.2637

6 Kahn JM, Davis BS, Yabes JG *et al.* Association Between State-Mandated Protocolized Sepsis Care and In-hospital Mortality Among Adults With Sepsis. *JAMA* 2019;**322**:240. doi:10.1001/jama.2019.9021

7 Lewis JM, Feasey NA, Rylance J. Aetiology and outcomes of sepsis in adults in sub-Saharan Africa: a systematic review and meta-analysis. *Critical Care* 2019;**23**:212. doi:10.1186/s13054-019-2501-y

8 Maitland K, Kiguli S, Opoka RO *et al.* Mortality after fluid bolus in African children with severe infection. *The New England journal of medicine* 2011;**364**:2483–95. doi:10.1056/NEJMoa1101549

9 Moore CC, Jacob ST, Banura P *et al.* Etiology of Sepsis in Uganda using a Quantitative PCR-based TaqMan Array Card. *Clin Infect Dis* 2019;**68**:266–72. doi:10.1093/cid/ciy472

10 Prasad N, Murdoch DR, Reyburn H *et al.* Etiology of Severe Febrile Illness in Low- and Middle-Income Countries: A Systematic Review. *PloS one* 2015;**10**:e0127962. doi:10.1371/journal.pone.0127962

11 Musicha P, Cornick JE, Bar-Zeev N *et al.* Trends in antimicrobial resistance in bloodstream infection isolates at a large urban hospital in Malawi (1998–2016): a surveillance study. *The Lancet Infectious Diseases* 2017;**17**:1042–52. doi:10.1016/S1473-3099(17)30394-8

12 Iroh Tam P-Y, Musicha P, Kawaza K *et al.* Emerging Resistance to Empiric Antimicrobial Regimens for Pediatric Bloodstream Infections in Malawi (1998–2017). *Clinical Infectious Diseases* 2019;**69**:61–8. doi:10.1093/cid/ciy834

13 Jacob ST, Moore CC, Banura P *et al.* Severe sepsis in two Ugandan hospitals: a prospective observational study of management and outcomes in a predominantly HIV-1 infected population. *PLoS One* 2009;**4**:e7782. doi:10.1371/journal.pone.0007782

14 Waitt PI, Mukaka M, Goodson P *et al.* Sepsis carries a high mortality among hospitalised adults in Malawi in the era of antiretroviral therapy scale-up: a longitudinal cohort study. *The Journal of infection* 2015;**70**:11–9. doi:10.1016/j.jinf.2014.07.004

15 Ssekitoleko R, Jacob ST, Banura P *et al.* Hypoglycemia at admission is associated with inhospital mortality in Ugandan patients with severe sepsis. *Crit Care Med* 2011;**39**:2271–6. doi:10.1097/CCM.0b013e3182227bd2

16 Ssekitoleko R, Pinkerton R, Muhindo R *et al.* Aggregate evaluable organ dysfunction predicts in-hospital mortality from sepsis in Uganda. *Am J Trop Med Hyg* 2011;**85**:697–702. doi:10.4269/ajtmh.2011.10-0692

17 Chimese SM, Andrews B, Lakhi S. The Etiology And Outcome Of Adult Patients Presenting With Sepsis To The University Teaching Hospital, Lusaka, Zambia. *Med J Zambia* 2012;**39**:19–22.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5663186/pdf/nihms524771.pdf

18 Moore CC, Hazard R, Saulters KJ *et al.* Derivation and validation of a universal vital assessment (UVA) score: a tool for predicting mortality in adult hospitalised patients in sub-Saharan Africa. *BMJ Glob Health* 2017;**2**:e000344. doi:10.1136/bmjgh-2017-000344

19 Huson MAM, Kalkman R, Stolp SM *et al.* The impact of HIV on presentation and outcome of bacterial sepsis and other causes of acute febrile illness in Gabon. *Infection* 2015;**43**:443–51. doi:10.1007/s15010-015-0753-2

20 Amir A, Saulters KJ, Muhindo R *et al.* Outcomes of patients with severe infection in Uganda according to adherence to the World Health Organization's Integrated Management of Adolescent and Adult Illness fluid resuscitation guidelines. *J Crit Care* 2017;**41**:24–8.

doi:10.1016/j.jcrc.2017.04.042

21 Andrews B, Muchemwa L, Kelly P *et al.* Simplified severe sepsis protocol: a randomized controlled trial of modified early goal-directed therapy in Zambia. *Crit Care Med* 2014;**42**:2315–24. doi:10.1097/ccm.0000000000000541

22 Andrews B, Semler MW, Muchemwa L *et al.* Effect of an Early Resuscitation Protocol on In-hospital Mortality Among Adults With Sepsis and Hypotension. *JAMA* 2017;**318**:1233. doi:10.1001/jama.2017.10913

23 Jacob ST, Banura P, Baeten JM *et al.* The impact of early monitored management on survival in hospitalized adult Ugandan patients with severe sepsis: a prospective intervention study*. *Crit Care Med* 2012;**40**:2050–8. doi:10.1097/CCM.0b013e31824e65d7

24 Zida S, Kania D, Sotto A *et al.* Leptospirosis as Cause of Febrile Icteric Illness, Burkina Faso. *Emerging Infectious Diseases* 2018;**24**:1569–72. doi:10.3201/eid2408.170436

25 Guillebaud J, Bernardson B, Randriambolamanantsoa TH *et al.* Study on causes of fever in primary healthcare center uncovers pathogens of public health concern in Madagascar. *PLOS Neglected Tropical Diseases* 2018;**12**:e0006642. doi:10.1371/journal.pntd.0006642

26 Maze MJ, Cash-Goldwasser S, Rubach MP *et al.* Risk factors for human acute leptospirosis in northern Tanzania. *PLOS Neglected Tropical Diseases* 2018;**12**:e0006372. doi:10.1371/journal.pntd.0006372

27 Gadia CLB, Manirakiza A, Tekpa G *et al.* Identification of pathogens for differential diagnosis of fever with jaundice in the Central African Republic: a retrospective assessment, 2008–2010. *BMC Infectious Diseases* 2017;**17**:735. doi:10.1186/s12879-017-2840-8

28 Hagen RM, Frickmann H, Ehlers J *et al.* Presence of Borrelia spp. DNA in ticks, but absence of Borrelia spp. and of Leptospira spp. DNA in blood of fever patients in Madagascar. *Acta Tropica* 2018;**177**:127–34. doi:10.1016/j.actatropica.2017.10.002

29 Biscornet L, Dellagi K, Pagès F *et al.* Human leptospirosis in Seychelles: A prospective study confirms the heavy burden of the disease but suggests that rats are not the main reservoir. *PLOS Neglected Tropical Diseases* 2017;**11**:e0005831. doi:10.1371/journal.pntd.0005831

30 Dreyfus A, Dyal JW, Pearson R *et al.* Leptospira Seroprevalence and Risk Factors in Health Centre Patients in Hoima District, Western Uganda. *PLOS Neglected Tropical Diseases* 2016;**10**:e0004858. doi:10.1371/journal.pntd.0004858

31 Hercik C, Cosmas L, Mogeni OD *et al.* A diagnostic and epidemiologic investigation of acute febrile illness (AFI) in Kilombero, Tanzania. *PLOS ONE* 2017;**12**:e0189712. doi:10.1371/journal.pone.0189712

32 Chipwaza B, Mugasa JP, Selemani M *et al.* Dengue and Chikungunya Fever among Viral Diseases in Outpatient Febrile Children in Kilosa District Hospital, Tanzania. *PLoS Neglected Tropical Diseases* 2014;**8**:e3335. doi:10.1371/journal.pntd.0003335

33 Cash-Goldwasser S, Crump JA, Halliday JEB *et al.* Risk Factors for Human Brucellosis in Northern Tanzania. *The American Journal of Tropical Medicine and Hygiene* 2018;**98**:598–606. doi:10.4269/ajtmh.17-0125

34 Gafirita J, Njunwa KJ, Ruhirwa R *et al.* Seroprevalence of Brucellosis among Patients Attending a District Hospital in Rwanda. *The American Journal of Tropical Medicine and Hygiene* 2017;**97**:831–5. doi:10.4269/ajtmh.16-0632

35 Boone I, Henning K, Hilbert A *et al.* Are brucellosis, Q fever and melioidosis potential causes of febrile illness in Madagascar? *Acta Tropica* 2017;**172**:255–62. doi:10.1016/j.actatropica.2017.05.013

36 Glanville WA de, Conde-Álvarez R, Moriyón I *et al.* Poor performance of the rapid test for human brucellosis in health facilities in Kenya. *PLOS Neglected Tropical Diseases* 2017;**11**:e0005508. doi:10.1371/journal.pntd.0005508

37 Njeru J, Melzer F, Wareth G *et al.* Human Brucellosis in Febrile Patients Seeking Treatment at Remote Hospitals, Northeastern Kenya, 2014-2015. *Emerging infectious diseases* 2016;**22**:2160–4. doi:10.3201/eid2212.160285

38 Chipwaza B, Mhamphi GG, Ngatunga SD *et al.* Prevalence of Bacterial Febrile Illnesses in Children in Kilosa District, Tanzania. *PLOS Neglected Tropical Diseases* 2015;**9**:e0003750. doi:10.1371/journal.pntd.0003750

39 Feleke SM, Animut A, Belay M. Prevalence of Malaria among Acute Febrile Patients Clinically Suspected of Having Malaria in the Zeway Health Center, Ethiopia. *Japanese Journal of Infectious Diseases* 2015;**68**:55–9. doi:10.7883/yoken.JJID.2013.062

40 Amoako N, Duodu S, Dennis FE *et al.* Detection of Dengue Virus among Children with Suspected Malaria, Accra, Ghana. *Emerging Infectious Diseases* 2018;**24**:1544–7. doi:10.3201/eid2408.180341

41 Njeru J, Henning K, Pletz MW *et al.* Febrile patients admitted to remote hospitals in Northeastern Kenya: seroprevalence, risk factors and a clinical prediction tool for Q-Fever. *BMC Infectious Diseases* 2016;**16**:244. doi:10.1186/s12879-016-1569-0

42 Mourembou G, Nzondo SM, Ndjoyi-Mbiguino A *et al.* Co-circulation of Plasmodium and Bacterial DNAs in Blood of Febrile and Afebrile Children from Urban and Rural Areas in Gabon. *The American Journal of Tropical Medicine and Hygiene* 2016;**95**:123–32.

doi:10.4269/ajtmh.15-0751

43 Angelakis E, Mediannikov O, Socolovschi C *et al.* Coxiella burnetii-positive PCR in febrile patients in rural and urban Africa. *International Journal of Infectious Diseases* 2014;**28**:107–10. doi:10.1016/j.ijid.2014.05.029

44 Maina AN, Farris CM, Odhiambo A *et al.* Q Fever, Scrub Typhus, and Rickettsial Diseases in Children, Kenya, 2011–2012. *Emerging Infectious Diseases* 2016;**22**:883–6. doi:10.3201/eid2205.150953

45 Sothmann P, Keller C, Krumkamp R *et al.* Rickettsia felis Infection in Febrile Children, Ghana. *The American Journal of Tropical Medicine and Hygiene* 2017;**96**:16–0754. doi:10.4269/ajtmh.16-0754

46 Elfving K, Shakely D, Andersson M *et al.* Acute Uncomplicated Febrile Illness in Children Aged 2-59 months in Zanzibar – Aetiologies, Antibiotic Treatment and Outcome. *PLOS ONE* 2016;**11**:e0146054. doi:10.1371/journal.pone.0146054

47 Mourembou G, Lekana-Douki JB, Mediannikov O *et al.* Possible Role of Rickettsia felis in Acute Febrile Illness among Children in Gabon. *Emerging Infectious Diseases* 2015;**21**:1808–15. doi:10.3201/eid2110.141825

48 Vu DM, Mutai N, Heath CJ *et al.* Unrecognized Dengue Virus Infections in Children, Western Kenya, 2014-2015. *Emerging infectious diseases* 2017;**23**:1915–7. doi:10.3201/eid2311.170807

49 Waggoner J, Brichard J, Mutuku F *et al.* Malaria and Chikungunya Detected Using Molecular Diagnostics Among Febrile Kenyan Children. *Open Forum Infectious Diseases* 2017;**4**:ofx110. doi:10.1093/ofid/ofx110

50 Kolawole OM, Seriki AA, Irekeola AA *et al.* Dengue virus and malaria concurrent infection among febrile subjects within Ilorin metropolis, Nigeria. *Journal of Medical Virology* 2017;**89**:1347–53. doi:10.1002/jmv.24788

51 Nasir IA, Agbede OO, Dangana A *et al.* Dengue virus non-structural Protein-1 expression and associated risk factors among febrile Patients attending University of Abuja Teaching Hospital, Nigeria. *Virus Research* 2017;**230**:7–12. doi:10.1016/j.virusres.2016.12.011

52 Ngoi CN, Price MA, Fields B *et al.* Dengue and Chikungunya Virus Infections among Young Febrile Adults Evaluated for Acute HIV-1 Infection in Coastal Kenya. *PLOS ONE* 2016;**11**:e0167508. doi:10.1371/journal.pone.0167508

53 Onoja A, Adeniji J, Olaleye O. High rate of unrecognized dengue virus infection in parts of the rainforest region of Nigeria. *Acta Tropica* 2016;**160**:39–43.

doi:10.1016/j.actatropica.2016.04.007

54 Kajeguka DC, Kaaya RD, Mwakalinga S *et al.* Prevalence of dengue and chikungunya virus infections in north-eastern Tanzania: a cross sectional study among participants presenting with malaria-like symptoms. *BMC Infectious Diseases* 2016;**16**:183. doi:10.1186/s12879-016-1511-5

55 Sow A, Loucoubar C, Diallo D *et al.* Concurrent malaria and arbovirus infections in Kedougou, southeastern Senegal. *Malaria Journal* 2016;**15**:47. doi:10.1186/s12936-016-1100-5

56 Kayiwa JT, Nankya AM, Ataliba IJ *et al.* Confirmation of Zika virus infection through hospital-based sentinel surveillance of acute febrile illness in Uganda, 2014–2017. *Journal of General Virology* Published Online First: July 2018. doi:10.1099/jgv.0.001113

57 Makiala-Mandanda S, Ahuka-Mundeke S, Abbate JL *et al.* Identification of Dengue and Chikungunya Cases Among Suspected Cases of Yellow Fever in the Democratic Republic of the Congo. *Vector-Borne and Zoonotic Diseases* 2018;**18**:364–70. doi:10.1089/vbz.2017.2176

58 Muianga A, Pinto G, Massangaie M *et al.* Antibodies Against Chikungunya in Northern Mozambique During Dengue Outbreak, 2014. *Vector-Borne and Zoonotic Diseases* 2018;vbz.2017.2261. doi:10.1089/vbz.2017.2261

59 Mugabe VA, Ali S, Chelene I *et al.* Evidence for chikungunya and dengue transmission in Quelimane, Mozambique: Results from an investigation of a potential outbreak of chikungunya virus. *PloS one* 2018;**13**:e0192110. doi:10.1371/journal.pone.0192110

60 António VS, Muianga AF, Wieseler J *et al.* Seroepidemiology of Chikungunya Virus Among Febrile Patients in Eight Health Facilities in Central and Northern Mozambique, 2015–2016. *Vector-Borne and Zoonotic Diseases* 2018;**18**:311–6. doi:10.1089/vbz.2017.2227

61 Sow A, Faye O, Diallo M *et al.* Chikungunya Outbreak in Kedougou, Southeastern Senegal in 2009–2010. *Open Forum Infectious Diseases* 2018;**5**:ofx259. doi:10.1093/ofid/ofx259

62 Olajiga OM, Adesoye OE, Emilolorun AP *et al.* Chikungunya Virus Seroprevalence and Associated Factors among Hospital Attendees in Two States of Southwest Nigeria: A Preliminary Assessment. *Immunological Investigations* 2017;**46**:552–65. doi:10.1080/08820139.2017.1319383

63 Bahr NC, Sarosi GA, Meya DB *et al.* Seroprevalence of histoplasmosis in Kampala, Uganda. *Medical Mycology* 2016;**54**:295–300. doi:10.1093/mmy/myv081

64 Mandengue CE, Ngandjio A, Atangana PJ. Histoplasmosis in HIV-Infected Persons, Yaoundé, Cameroon. *Emerging Infectious Diseases* 2015;**21**:2094–6. doi:10.3201/eid2111.150278

65 Schwarzer G. meta: An R package for meta-analysis. *R News* 2007;**7**:40–5.

66 Bone RC, Balk RA, Cerra FB *et al.* Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992;**101**:1644–55.http://www.ncbi.nlm.nih.gov/pubmed/1303622

67 Levy MM, Fink MP, Marshall JC *et al.* 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Critical Care Medicine* 2003;**31**:1250–6. doi:10.1097/01.CCM.0000050454.01978.3B

68 Vincent JL, Mendonça A de, Cantraine F *et al.* Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. *Critical care medicine* 1998;**26**:1793–800.http://www.ncbi.nlm.nih.gov/pubmed/9824069

69 Seymour CW, Liu VX, Iwashyna TJ *et al.* Assessment of Clinical Criteria for Sepsis. *JAMA* 2016;**315**:762. doi:10.1001/jama.2016.0288

70 Shankar-Hari M, Phillips GS, Levy ML *et al.* Developing a New Definition and Assessing New Clinical Criteria for Septic Shock. *JAMA* 2016;**315**:775. doi:10.1001/jama.2016.0289

71 Jacob ST, West TE, Banura P. Fitting a square peg into a round hole: are the current Surviving Sepsis Campaign guidelines feasible for Africa? *Crit Care* 2011;**15**:117. doi:10.1186/cc9981

72 Prin M, Itaye T, Clark S *et al.* Critical Care in a Tertiary Hospital in Malawi. *World J Surg* 2016;**40**:2635–42. doi:10.1007/s00268-016-3578-y

73 Huson MA, Kalkman R, Grobusch MP *et al.* Predictive value of the qSOFA score in patients with suspected infection in a resource limited setting in Gabon. *Travel Med Infect Dis* 2017;**15**:76–7. doi:10.1016/j.tmaid.2016.10.014

74 Huson MAM, Katete C, Chunda L *et al.* Application of the qSOFA score to predict mortality in patients with suspected infection in a resource-limited setting in Malawi. *Infection* 2017;**45**:893–6. doi:10.1007/s15010-017-1057-5

75 Aluisio AR, Garbern S, Wiskel T *et al.* Mortality outcomes based on ED qSOFA score and HIV status in a developing low income country. *The American Journal of Emergency Medicine* Published Online First: March 2018. doi:10.1016/j.ajem.2018.03.014

76 Rudd KE, Seymour CW, Aluisio AR *et al.* Association of the Quick Sequential (Sepsis-Related) Organ Failure Assessment (qSOFA) Score With Excess Hospital Mortality in Adults With Suspected Infection in Low- and Middle-Income Countries. *JAMA* 2018;**319**:2202. doi:10.1001/jama.2018.6229

77 Gaieski DF, Edwards JM, Kallan MJ *et al.* Benchmarking the Incidence and Mor-

tality of Severe Sepsis in the United States*. *Critical Care Medicine* 2013;**41**:1167–74. doi:10.1097/CCM.0b013e31827c09f8

78 Angus DC, Linde-Zwirble WT, Lidicker J *et al.* Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Critical care medicine* 2001;**29**:1303–10.http://www.ncbi.nlm.nih.gov/pubmed/11445675

79 Tsertsvadze A, Royle P, Seedat F *et al.* Community-onset sepsis and its public health burden: a systematic review. *Systematic reviews* 2016;**5**:81. doi:10.1186/s13643-016-0243-3

80 Mayr FB, Yende S, Linde-Zwirble WT *et al.* Infection Rate and Acute Organ Dysfunction Risk as Explanations for Racial Differences in Severe Sepsis. *JAMA* 2010;**303**:2495. doi:10.1001/jama.2010.851

81 Seymour CW, Gesten F, Prescott HC *et al.* Time to Treatment and Mortality during Mandated Emergency Care for Sepsis. *New England Journal of Medicine* 2017;**376**:2235–44. doi:10.1056/NEJMoa1703058

82 PRISM Investigators, Rowan KM, Angus DC *et al.* Early, Goal-Directed Therapy for Septic Shock — A Patient-Level Meta-Analysis. *New England Journal of Medicine* 2017;**376**:2223–34. doi:10.1056/NEJMoa1701380

83 Rangel-Frausto MS, Pittet D, Costigan M *et al.* The Natural History of the Systemic Inflammatory Response Syndrome (SIRS). *JAMA* 1995;**273**:117. doi:10.1001/jama.1995.03520260039030

84 Serafim R, Gomes JA, Salluh J *et al.* A Comparison of the Quick-SOFA and Systemic Inflammatory Response Syndrome Criteria for the Diagnosis of Sepsis and Prediction of Mortality. *Chest* 2018;**153**:646–55. doi:10.1016/j.chest.2017.12.015

85 Stevenson EK, Rubenstein AR, Radin GT *et al.* Two Decades of Mortality Trends Among Patients With Severe Sepsis. *Critical Care Medicine* 2014;**42**:625–31. doi:10.1097/CCM.0000000000000026

86 Winters BD, Eberlein M, Leung J *et al.* Long-term mortality and quality of life in sepsis: A systematic review*. *Critical Care Medicine* 2010;**38**:1276–83. doi:10.1097/CCM.0b013e3181d8cc1d

87 Shah FA, Pike F, Alvarez K *et al.* Bidirectional Relationship between Cognitive Function and Pneumonia. *American Journal of Respiratory and Critical Care Medicine* 2013;**188**:586–92. doi:10.1164/rccm.201212-2154OC

88 Iwashyna TJ, Ely EW, Smith DM *et al.* Long-term Cognitive Impairment and Functional Disability Among Survivors of Severe Sepsis. *JAMA* 2010;**304**:1787. doi:10.1001/jama.2010.1553

89 Yende S, Linde-Zwirble W, Mayr F *et al.* Risk of cardiovascular events in survivors of severe sepsis. *American journal of respiratory and critical care medicine* 2014;**189**:1065–74. doi:10.1164/rccm.201307-1321OC

90 Bergh C, Fall K, Udumyan R *et al.* Severe infections and subsequent delayed cardiovascular disease. *European journal of preventive cardiology* 2017;**24**:1958–66. doi:10.1177/2047487317724009

91 Ou S-M, Chu H, Chao P-W *et al.* Long-Term Mortality and Major Adverse Cardiovascular Events in Sepsis Survivors. A Nationwide Population-based Study. *American Journal of Respiratory and Critical Care Medicine* 2016;**194**:209–17. doi:10.1164/rccm.201510-2023OC

92 Vugia DJ, Kiehlbauch JA, Yeboue K *et al.* Pathogens and predictors of fatal septicemia associated with human immunodeficiency virus infection in Ivory Coast, west Africa. *J Infect Dis* 1993;**168**:564–70.http://jid.oxfordjournals.org/content/168/3/564.full.pdf

93 Archibald LK, Dulk MO den, Pallangyo KJ *et al.* Fatal Mycobacterium tuberculosis bloodstream infections in febrile hospitalized adults in Dar es Salaam, Tanzania. *Clin Infect Dis* 1998;**26**:290–6.http://cid.oxfordjournals.org/content/26/2/290.full.pdf

94 Ssali FN. A prospective study of community-Acquired Bloodstream infections Amoung ferbrile Adults Admitted to Mulago Hospital. 1998.

95 McDonald LC, Archibald LK, Rheanpumikankit S *et al.* Unrecognised Mycobacterium tuberculosis bacteraemia among hospital inpatients in less developed countries. *Lancet* 1999;**354**:1159–63.http://ac.els-cdn.com/S0140673698123255/1-s2.0-S0140673698123255-main.pdf?{_}tid=f55329a2-a257-11e5-8ca0-00000aab0f01{\&}acdnat=1450093616{_}bee18e675c9101d403259

96 Archibald LK, McDonald LC, Nwanyanwu O *et al.* A hospital-based prevalence survey of bloodstream infections in febrile patients in Malawi: implications for diagnosis and therapy. *J Infect Dis* 2000;**181**:1414–20. doi:10.1086/315367

97 Bell M, Archibald LK, Nwanyanwu O *et al.* Seasonal variation in the etiology of bloodstream infections in a febrile inpatient population in a developing country. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases* 2001;**5**:63–9. doi:10.1016/S1201-9712(01)90027-X

98 Lewis DK, Peters RPH, Schijffelen MJ *et al.* Clinical indicators of mycobacteraemia in adults admitted to hospital in Blantyre, Malawi. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2002;**6**:1067–74.http://www.ncbi.nlm.nih.gov/pubmed/12546114

99 Crump JA, Morrissey AB, Nicholson WL *et al.* Etiology of Severe Non-malaria Febrile

Illness in Northern Tanzania: A Prospective Cohort Study. *PLoS Neglected Tropical Diseases* 2013;**7**:e2324. doi:10.1371/journal.pntd.0002324

100 Gilks CF, Brindle RJ, Mwachari C *et al.* Disseminated Mycobacterium avium infection among HIV-infected patients in Kenya. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology* 1995;**8**:195–8.http://www.scopus.com/inward/record.url?eid=2-s2.0-0028938853{\&}partnerID=40{\&}md5=38dc87e275e9e7f14ff3a593cfeba69b

101 Gottberg A von, Sacks L, Machala S *et al.* Utility of blood cultures and incidence of mycobacteremia in patients with suspected tuberculosis in a South African infectious disease referral hospital. *Int J Tuberc Lung Dis* 2001;**5**:80–6.

102 Munseri PJ, Talbot E a, Bakari M *et al.* The bacteraemia of disseminated tuberculosis among HIV-infected patients with prolonged fever in Tanzania. *Scandinavian journal of infectious diseases* 2011;**43**:696–701. doi:10.3109/00365548.2011.577802

103 Nakiyingi L, Ssengooba W, Nakanjako D *et al.* Predictors and outcomes of mycobacteremia among HIV-infected smear- negative presumptive tuberculosis patients in Uganda. *BMC Infectious Diseases* 2015;**15**. doi:10.1186/s12879-015-0812-4

104 Lawn SD, Kerkhoff AD, Burton R *et al.* Rapid microbiological screening for tuberculosis in HIV-positive patients on the first day of acute hospital admission by systematic testing of urine samples using Xpert MTB/RIF: a prospective cohort in South Africa. *BMC medicine* 2015;**13**:192. doi:10.1186/s12916-015-0432-2

105 Gupta-Wright A, Corbett EL, Oosterhout JJ van *et al.* Rapid urine-based screening for tuberculosis in HIV-positive patients admitted to hospital in Africa (STAMP): a pragmatic, multicentre, parallel-group, double-blind, randomised controlled trial. *Lancet (London, England)* 2018;**392**:292–301. doi:10.1016/S0140-6736(18)31267-4

106 Gupta RK, Lucas SB, Fielding KL *et al.* Prevalence of tuberculosis in post-mortem studies of HIV-infected adults and children in resource-limited settings. *AIDS* 2015;**29**:1987–2002. doi:10.1097/QAD.0000000000000802

107 World Health Organisation. Improving the diagnosis and treatment of smear-negative pulmonary and extrapulmonary tuberculosis among adults and adolescents: recommendations for HIV-prevalent and resource-constrained settings. 2007.

108 Holtz TH, Kabera G, Mthiyane T *et al.* Use of a WHO-recommended algorithm to reduce mortality in seriously ill patients with HIV infection and smear-negative pulmonary tuberculosis in South Africa: an observational cohort study. *The Lancet Infectious diseases* 2011;**11**:533–40. doi:10.1016/S1473-3099(11)70057-3

109 Osawa R, Singh N. Cytomegalovirus infection in critically ill patients: a systematic review. *Critical Care* 2009;**13**:R68. doi:10.1186/cc7875

110 Rubach MP, Maro VP, Bartlett JA *et al.* Etiologies of illness among patients meeting integrated management of adolescent and adult illness district clinician manual criteria for severe infections in northern Tanzania: implications for empiric antimicrobial therapy. *Am J Trop Med Hyg* 2015;**92**:454–62. doi:10.4269/ajtmh.14-0496

111 World Health Organisation. IMAI district clinician manual: hospital care for adolescents and adults. 2011.

112 Rajasingham R, Smith RM, Park BJ *et al.* Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *The Lancet Infectious Diseases* 2017;**17**:873–81. doi:10.1016/S1473-3099(17)30243-8

113 Wasserman S, Engel ME, Mendelson M. Burden of pneumocystis pneumonia in HIV-infected adults in sub-Saharan Africa: protocol for a systematic review. *Syst Rev* 2013;**2**:112. doi:10.1186/2046-4053-2-112

114 Rhodes A, Evans LE, Alhazzani W *et al.* Surviving Sepsis Campaign. *Critical Care Medicine* 2017;**45**:486–552. doi:10.1097/CCM.0000000000002255

115 Dünser MW, Festic E, Dondorp A *et al.* Recommendations for sepsis management in resource-limited settings. *Intensive Care Medicine* 2012;**38**:557–74. doi:10.1007/s00134-012-2468-5

116 Mer M, Schultz MJ, Adhikari NK *et al.* Core elements of general supportive care for patients with sepsis and septic shock in resource-limited settings. *Intensive Care Medicine* 2017;**43**:1690–4. doi:10.1007/s00134-017-4831-z

117 Thwaites CL, Lundeg G, Dondorp AM *et al.* Recommendations for infection management in patients with sepsis and septic shock in resource-limited settings. *Intensive Care Medicine* 2016;**42**:2040–2. doi:10.1007/s00134-016-4415-3

118 Misango D, Pattnaik R, Baker T *et al.* Haemodynamic assessment and support in sepsis and septic shock in resource-limited settings. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2017;**111**:483–9. doi:10.1093/trstmh/try007

119 Rivers E, Nguyen B, Havstad S *et al.* Early Goal-Directed Therapy in the Treatment of Severe Sepsis and Septic Shock. *New England Journal of Medicine* 2001;**345**:1368–77. doi:10.1056/NEJMoa010307

120 Yealy DM, Kellum JA, Huang DT *et al.* A randomized trial of protocol-based care for early septic shock. *The New England journal of medicine* 2014;**370**:1683–93.

doi:10.1056/NEJMoa1401602

121 Peake SL, Delaney A, Bailey M *et al.* Goal-Directed Resuscitation for Patients with Early Septic Shock. *New England Journal of Medicine* 2014;**371**:141001063014008. doi:10.1056/NEJMoa1404380

122 Mouncey PR, Osborn TM, Power GS *et al.* Trial of Early, Goal-Directed Resuscitation for Septic Shock. *New England Journal of Medicine* 2015;**372**:150317011022003. doi:10.1056/NEJMoa1500896

123 Kumar A, Roberts D, Wood KE *et al.* Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Critical care medicine* 2006;**34**:1589–96. doi:10.1097/01.CCM.0000217961.75225.E9

124 Sterling SA, Miller WR, Pryor J *et al.* The Impact of Timing of Antibiotics on Outcomes in Severe Sepsis and Septic Shock. *Critical Care Medicine* 2015;**43**:1907–15. doi:10.1097/CCM.0000000000001142

125 Amir A, Saulters KJ, Olum S *et al.* Outcomes of patients with severe sepsis after the first 6 hours of resuscitation at a regional referral hospital in Uganda. *J Crit Care* 2016;**33**:78–83. doi:10.1016/j.jcrc.2016.01.023

126 Chalya PL, Mabula JB, Koy M *et al.* Typhoid intestinal perforations at a University teaching hospital in Northwestern Tanzania: A surgical experience of 104 cases in a resource-limited setting. *World journal of emergency surgery : WJES* 2012;**7**:4. doi:10.1186/1749-7922-7-4

127 Ferrer R, Artigas A, Suarez D *et al.* Effectiveness of Treatments for Severe Sepsis. *American Journal of Respiratory and Critical Care Medicine* 2009;**180**:861–6. doi:10.1164/rccm.200812-1912OC

128 Lee SJ, Ramar K, Park JG *et al.* Increased fluid administration in the first three hours of sepsis resuscitation is associated with reduced mortality: a retrospective cohort study. *Chest* 2014;**146**:908–15. doi:10.1378/chest.13-2702

129 Leisman DE, Goldman C, Doerfler ME *et al.* Patterns and Outcomes Associated With Timeliness of Initial Crystalloid Resuscitation in a Prospective Sepsis and Septic Shock Cohort*. *Critical Care Medicine* 2017;**45**:1596–606. doi:10.1097/CCM.0000000000002574

130 Leisman DE, Doerfler ME, Schneider SM *et al.* Predictors, Prevalence, and Outcomes of Early Crystalloid Responsiveness Among Initially Hypotensive Patients With Sepsis and Septic Shock*. *Critical Care Medicine* 2018;**46**:189–98. doi:10.1097/CCM.0000000000002834

131 Maitland K, George EC, Evans JA *et al.* Exploring mechanisms of excess mortality

with early fluid resuscitation: insightsfrom the FEAST trial. *BMC Medicine* 2013;**11**:68. doi:10.1186/1741-7015-11-68

132 Ambler RP. The Structure of beta-lactamases. *Philosophical Transactions of the Royal Society B: Biological Sciences* 1980;**289**:321–31. doi:10.1098/rstb.1980.0049

133 Bush K, Jacoby GA. Updated functional classification of beta-lactamases. *Antimicrobial agents and chemotherapy* 2010;**54**:969–76. doi:10.1128/AAC.01009-09

134 Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. *Clinical microbiology reviews* 2005;**18**:657–86. doi:10.1128/CMR.18.4.657-686.2005

135 Bradford PA. Extended-Spectrum-Lactamases in the 21st Century: Characterization, Epidemiology, and Detection of This Important Resistance Threat. *Clinical Microbiology and Infection* 2001;**14**:933–51. doi:10.1128/CMR.14.4.933-951.2001

136 ABRAHAM EP, CHAIN E. An Enzyme from Bacteria able to Destroy Penicillin. *Nature* 1940;**146**:837–7. doi:10.1038/146837a0

137 Datta N, Kontomichalou P. Penicillinase synthesis controlled by infectious R factors in Enterobacteriaceae. *Nature* 1965;**208**:239–41.http://www.ncbi.nlm.nih.gov/pubmed/5326330

138 Knothe H, Shah P, Krcmery V *et al.* Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of Klebsiella pneumoniae and Serratia marcescens. *Infection* 1983;**11**:315–7.http://www.ncbi.nlm.nih.gov/pubmed/6321357

139 Kliebe C, Nies BA, Meyer JF *et al.* Evolution of plasmid-coded resistance to broad-spectrum cephalosporins. *Antimicrobial agents and chemotherapy* 1985;**28**:302–7.http://www.ncbi.nlm.nih.gov/pubmed/3879659 http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=P

140 Liakopoulos A, Mevius D, Ceccarelli D. A Review of SHV Extended-Spectrum β-Lactamases: Neglected Yet Ubiquitous. *Frontiers in microbiology* 2016;**7**:1374. doi:10.3389/fmicb.2016.01374

141 Sougakoff W, Goussard S, Gerbaud G *et al.* Plasmid-mediated resistance to third-generation cephalosporins caused by point mutations in TEM-type penicillinase genes. *Reviews of infectious diseases* 1988;**10**:879–84.http://www.ncbi.nlm.nih.gov/pubmed/3055179

142 Gold HS, Moellering RC. Antimicrobial-Drug Resistance. *New England Journal of Medicine* 1996;**335**:1445–53. doi:10.1056/NEJM199611073351907

143 Philippon A, Labia R, Jacoby G. Extended-spectrum beta-lactamases. *Antimicrobial agents and chemotherapy* 1989;**33**:1131–6.http://www.ncbi.nlm.nih.gov/pubmed/2679367 http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC172613

144 Cantón R, Coque TM. The CTX-M beta-lactamase pandemic. *Current opinion in microbiology* 2006;**9**:466–75. doi:10.1016/j.mib.2006.08.011

145 Livermore DM, Canton R, Gniadkowski M *et al.* CTX-M: changing the face of ESBLs in Europe. *Journal of Antimicrobial Chemotherapy* 2006;**59**:165–74. doi:10.1093/jac/dkl483

146 Ben-Ami R, Rodríguez-Baño J, Arslan H *et al.* A Multinational Survey of Risk Factors for Infection with Extended-Spectrum β-Lactamase–Producing Enterobacteriaceae in Nonhospitalized Patients. *Clinical Infectious Diseases* 2009;**49**:682–90. doi:10.1086/604713

147 Bauernfeind A, Grimm H, Schweighart S. A new plasmidic cefotaximase in a clinical isolate of Escherichia coli. *Infection* 1990;**18**:294–8.http://www.ncbi.nlm.nih.gov/pubmed/2276823

148 Bonnet R. Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. *Antimicrobial agents and chemotherapy* 2004;**48**:1–14. doi:10.1128/AAC.48.1.1-14.2004

149 Bevan ER, Jones AM, Hawkey PM. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. *Journal of Antimicrobial Chemotherapy* 2017;**72**:2145–55. doi:10.1093/jac/dkx146

150 Karanika S, Karantanos T, Arvanitis M *et al.* Fecal Colonization With Extended-spectrum Beta-lactamase–Producing Enterobacteriaceae and Risk Factors Among Healthy Individuals: A Systematic Review and Metaanalysis. *Clinical Infectious Diseases* 2016;**63**:310–8. doi:10.1093/cid/ciw283

151 Goossens H, Ferech M, Vander Stichele R *et al.* Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. *The Lancet* 2005;**365**:579–87. doi:10.1016/S0140-6736(05)17907-0

152 Lai C-C, Wang C-Y, Chu C-C *et al.* Correlation between antibiotic consumption and resistance of Gram-negative bacteria causing healthcare-associated infections at a university hospital in Taiwan from 2000 to 2009. *Journal of Antimicrobial Chemotherapy* 2011;**66**:1374–82. doi:10.1093/jac/dkr103

153 Alvarez-Uria G, Gandra S, Laxminarayan R. Poverty and prevalence of antimicrobial resistance in invasive isolates. *International Journal of Infectious Diseases* 2016;**52**:59–61. doi:10.1016/J.IJID.2016.09.026

154 Denis B, Lafaurie M, Donay J-L *et al.* Prevalence, risk factors, and impact on clinical outcome of extended-spectrum beta-lactamase-producing Escherichia coli bacteraemia: a five-year study. *International Journal of Infectious Diseases* 2015;**39**:1–6. doi:10.1016/J.IJID.2015.07.010

155 Gorrie CL, Mirceta M, Wick RR *et al.* Antimicrobial-Resistant Klebsiella pneumoniae

Carriage and Infection in Specialized Geriatric Care Wards Linked to Acquisition in the Referring Hospital. *Clinical Infectious Diseases* 2018;**67**:161–70. doi:10.1093/cid/ciy027

156 Mirelis B, Navarro F, Miró E *et al.* Community Transmission of Extended-Spectrum ß-Lactamase. *Emerging Infectious Diseases* 2003;**9**:1024–5. doi:10.3201/eid0908.030094

157 Franiczek R, Sobieszczańska B, Grabowski M *et al.* Occurrence of extended-spectrum beta-lactamases among Escherichia coli isolates from hospitalized and healthy children. *Folia microbiologica* 2003;**48**:243–7.http://www.ncbi.nlm.nih.gov/pubmed/12800510

158 Woerther P-L, Burdet C, Chachaty E *et al.* Trends in human fecal carriage of extended-spectrum β-lactamases in the community: toward the globalization of CTX-M. *Clinical microbiology reviews* 2013;**26**:744–58. doi:10.1128/CMR.00023-13

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

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

161 Ny S, Löfmark S, Börjesson S *et al.* Community carriage of ESBL-producing Escherichia coli is associated with strains of low pathogenicity: a Swedish nationwide study. *Journal of Antimicrobial Chemotherapy* 2017;**72**:582–8. doi:10.1093/jac/dkw419

162 Valverde A, Coque TM, Sanchez-Moreno MP *et al.* Dramatic Increase in Prevalence of Fecal Carriage of Extended-Spectrum -Lactamase-Producing Enterobacteriaceae during Nonoutbreak Situations in Spain. *Journal of Clinical Microbiology* 2004;**42**:4769–75. doi:10.1128/JCM.42.10.4769-4775.2004

163 Li B, Sun J-Y, Liu Q-Z *et al.* High prevalence of CTX-M β-lactamases in faecal Escherichia coli strains from healthy humans in Fuzhou, China. *Scandinavian Journal of Infectious Diseases* 2011;**43**:170–4. doi:10.3109/00365548.2010.538856

164 Babu R, Kumar A, Karim S *et al.* Faecal carriage rate of extended-spectrum β-lactamase-producing Enterobacteriaceae in hospitalised patients and healthy asymptomatic individuals coming for health check-up. *Journal of Global Antimicrobial Resistance* 2016;**6**:150–3. doi:10.1016/j.jgar.2016.05.007

165 Reuland EA, Al Naiemi N, Kaiser AM *et al.* Prevalence and risk factors for carriage of ESBL-producing Enterobacteriaceae in Amsterdam. *The Journal of antimicrobial chemotherapy* 2016;**71**:1076–82. doi:10.1093/jac/dkv441

166 Woerther P-L, Andremont A, Kantele A. Travel-acquired ESBL-producing Enterobacteriaceae: impact of colonization at individual and community level. *Journal of travel medicine* 2017;**24**:S29–34. doi:10.1093/jtm/taw101

167 March A, Aschbacher R, Dhanji H *et al.* Colonization of residents and staff of a long-term-care facility and adjacent acute-care hospital geriatric unit by multiresistant bacteria. *Clinical Microbiology and Infection* 2010;**16**:934–44. doi:10.1111/J.1469-0691.2009.03024.X

168 Valverde A, Grill F, Coque TM *et al.* High rate of intestinal colonization with extended-spectrum-beta-lactamase-producing organisms in household contacts of infected community patients. *Journal of clinical microbiology* 2008;**46**:2796–9. doi:10.1128/JCM.01008-08

169 Duijkeren E van, Wielders CCH, Dierikx CM *et al.* Long-term Carriage of Extended-Spectrum β-Lactamase–Producing Escherichia coli and Klebsiella pneumoniae in the General Population in The Netherlands. *Clinical Infectious Diseases* 2018;**66**:1368–76. doi:10.1093/cid/cix1015

170 Alsterlund R, Carlsson B, Gezelius L *et al.* Multiresistant CTX-M-15 ESBL-producing Escherichia coli in southern Sweden: Description of an outbreak. *Scandinavian Journal of Infectious Diseases* 2009;**41**:410–5. doi:10.1080/00365540902896079

171 Zahar J, Lanternier F, Mechai F *et al.* Duration of colonisation by Enterobacteriaceae producing extended-spectrum β-lactamase and risk factors for persistent faecal carriage. *Journal of Hospital Infection* 2010;**75**:76–8. doi:10.1016/j.jhin.2009.11.010

172 Lohr IH, Rettedal S, Natas OB *et al.* Long-term faecal carriage in infants and intra-household transmission of CTX-M-15-producing Klebsiella pneumoniae following a nosocomial outbreak. *Journal of Antimicrobial Chemotherapy* 2013;**68**:1043–8. doi:10.1093/jac/dks502

173 Arcilla MS, Hattem JM van, Haverkate MR *et al.* Import and spread of extended-spectrum β-lactamase-producing Enterobacteriaceae by international travellers (COMBAT study): a prospective, multicentre cohort study. *The Lancet Infectious Diseases* 2017;**17**:78–85. doi:10.1016/S1473-3099(16)30319-X

174 Teunis PFM, Evers EG, Hengeveld PD *et al.* Time to acquire and lose carriership of ESBL/pAmpC producing E. coli in humans in the Netherlands. *PLOS ONE* 2018;**13**:e0193834. doi:10.1371/journal.pone.0193834

175 Lartigue M-F, Poirel L, Aubert D *et al.* In vitro analysis of ISEcp1B-mediated mobilization of naturally occurring beta-lactamase gene blaCTX-M of Kluyvera ascorbata. *Antimicrobial agents and chemotherapy* 2006;**50**:1282–6. doi:10.1128/AAC.50.4.1282-1286.2006

176 Cantón R, María González-Alba J, Galán JC *et al.* CTX-M enzymes: origin and diffusion.

Published Online First: 2012. doi:10.3389/fmicb.2012.00110

177 Zhao W-H, Hu Z-Q. Epidemiology and genetics of CTX-M extended-spectrum β-lactamases in Gram-negative bacteria. *Critical reviews in microbiology* 2013;**39**:79–101. doi:10.3109/1040841X.2012.691460

178 Coque TM, Novais Â, Carattoli A *et al.* Dissemination of Clonally Related Escherichia coli Strains Expressing Extended-Spectrum β-Lactamase CTX-M-15. *Emerging Infectious Diseases* 2008;**14**:195–200. doi:10.3201/eid1402.070350

179 Nicolas-Chanoine M-H, Bertrand X, Madec J-Y. Escherichia coli ST131, an intriguing clonal group. *Clinical microbiology reviews* 2014;**27**:543–74. doi:10.1128/CMR.00125-13

180 Stoesser N, Sheppard AE, Pankhurst L *et al.* Evolutionary History of the Global Emergence of the Escherichia coli Epidemic Clone ST131. *mBio* 2016;**7**:e02162. doi:10.1128/mBio.02162-15

181 Petty NK, Ben Zakour NL, Stanton-Cook M *et al.* Global dissemination of a multidrug resistant Escherichia coli clone. *Proceedings of the National Academy of Sciences* 2014;**111**:5694–9. doi:10.1073/pnas.1322678111

182 Bates D, Mächler M, Bolker B *et al.* Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 2015;**67**:1–48. doi:10.18637/jss.v067.i01

183 Guiral E, Pons MJ, Vubil D *et al.* Epidemiology and molecular characterization of multidrug-resistant Escherichia coli isolates harboring blaCTX-M group 1 extended-spectrum beta-lactamases causing bacteremia and urinary tract infection in Manhica, Mozambique. *Infect Drug Resist* 2018;**11**:927–36. doi:10.2147/idr.s153601

184 Karppinen M, Bernardino L, Anjos ED *et al.* Etiology of Childhood Otorrhea in Luanda, Angola, and a Review of Otitis Media in African Children. *Pediatr Infect Dis J* Published Online First: 2018. doi:10.1097/inf.0000000000002254

185 Ibrahim Y, Sani Y, Saleh Q *et al.* Phenotypic Detection of Extended Spectrum Beta lactamase and Carbapenemase Co-producing Clinical Isolates from Two Tertiary Hospitals in Kano, North West Nigeria. *Ethiop J Health Sci* 2017;**27**:3–10.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5390223/pdf/EJHS2701-0003.pdf

186 Kassam NA, Damian DJ, Kajeguka D *et al.* Spectrum and antibiogram of bacteria isolated from patients presenting with infected wounds in a Tertiary Hospital, northern Tanzania. *BMC Res Notes* 2017;**10**:757. doi:10.1186/s13104-017-3092-9

187 Legese MH, Weldearegay GM, Asrat D. Extended-spectrum beta-lactamase- and carbapenemase-producing Enterobacteriaceae among Ethiopian children. *Infect Drug Resist*

2017;**10**:27–34. doi:10.2147/idr.s127177

188 Manyahi J, Moyo SJ, Tellevik MG *et al.* Detection of CTX-M-15 beta-lactamases in Enterobacteriaceae causing hospital- and community-acquired urinary tract infections as early as 2004, in Dar es Salaam, Tanzania. *BMC Infect Dis* 2017;**17**:282. doi:10.1186/s12879-017-2395-8

189 Sangare SA, Rondinaud E, Maataoui N *et al.* Very high prevalence of extended-spectrum beta-lactamase-producing Enterobacteriaceae in bacteriemic patients hospitalized in teaching hospitals in Bamako, Mali. *PLoS One* 2017;**12**. doi:10.1371/journal.pone.0172652

190 Abera B, Kibret M, Mulu W. Extended-Spectrum beta (beta)-Lactamases and Antibiogram in Enterobacteriaceae from Clinical and Drinking Water Sources from Bahir Dar City, Ethiopia. *PLoS One* 2016;**11**:e0166519. doi:10.1371/journal.pone.0166519

191 Agyekum A, Fajardo-Lubian A, Ansong D *et al.* blaCTX-M-15 carried by IncF-type plasmids is the dominant ESBL gene in Escherichia coli and Klebsiella pneumoniae at a hospital in Ghana. *Diagn Microbiol Infect Dis* 2016;**84**:328–33. doi:10.1016/j.diagmicrobio.2015.12.010

192 Breurec S, Bouchiat C, Sire JM *et al.* High third-generation cephalosporin resistant Enterobacteriaceae prevalence rate among neonatal infections in Dakar, Senegal. *BMC Infect Dis* 2016;**16**. doi:10.1186/s12879-016-1935-y

193 Buys H, Muloiwa R, Bamford C *et al.* Klebsiella pneumoniae bloodstream infections at a South African children's hospital 2006-2011, a cross-sectional study. *BMC Infect Dis* 2016;**16**:570. doi:10.1186/s12879-016-1919-y

194 Eibach D, Belmar Campos C, Krumkamp R *et al.* Extended spectrum beta-lactamase producing Enterobacteriaceae causing bloodstream infections in rural Ghana, 2007-2012. *Int J Med Microbiol* 2016;**306**:249–54. doi:10.1016/j.ijmm.2016.05.006

195 Kpoda DS, Ajayi A, Somda M *et al.* Distribution of resistance genes encoding ESBLs in Enterobacteriaceae isolated from biological samples in health centers in Ouagadougou, Burkina Faso. *BMC Res Notes* 2018;**11**:471. doi:10.1186/s13104-018-3581-5

196 Kabwe M, Tembo J, Chilukutu L *et al.* Etiology, antibiotic resistance and risk factors for neonatal sepsis in a large referral center in Zambia. *Pediatric Infectious Disease Journal* 2016;**35**:e191–8. doi:10.1097/INF.0000000000001154

197 Leski TA, Taitt CR, Bangura U *et al.* High prevalence of multidrug resistant Enterobacteriaceae isolated from outpatient urine samples but not the hospital environment in Bo, Sierra Leone. *BMC Infect Dis* 2016;**16**:167. doi:10.1186/s12879-016-1495-1

198 Mohammed Y, Gadzama GB, Zailani SB *et al.* Characterization of Extended-Spectrum

Beta-lactamase from Escherichia coli and Klebsiella Species from North Eastern Nigeria. *J Clin Diagn Res* 2016;**10**:Dc07–10. doi:10.7860/jcdr/2016/16330.7254

199 Naas T, Cuzon G, Robinson AL *et al.* Neonatal infections with multidrug-resistant ESBL-producing E. cloacae and K. pneumoniae in Neonatal Units of two different Hospitals in Antananarivo, Madagascar. *BMC Infect Dis* 2016;**16**:275. doi:10.1186/s12879-016-1580-5

200 Ndir A, Diop A, Faye PM *et al.* Epidemiology and Burden of Bloodstream Infections Caused by Extended-Spectrum Beta-Lactamase Producing Enterobacteriaceae in a Pediatric Hospital in Senegal. *PLoS One* 2016;**11**:e0143729. doi:10.1371/journal.pone.0143729

201 Ouedraogo AS, Sanou M, Kissou A *et al.* High prevalence of extended-spectrum ss-lactamase producing enterobacteriaceae among clinical isolates in Burkina Faso. *BMC Infect Dis* 2016;**16**:326. doi:10.1186/s12879-016-1655-3

202 Sangare SA, Maiga AI, Guindo I *et al.* Prevalence of ESBL-producing Enterobacteriaceae isolated from blood cultures in Mali. *J Infect Dev Ctries* 2016;**10**:1059–64. doi:10.3855/jidc.7536

203 Seni J, Falgenhauer L, Simeo N *et al.* Multiple ESBL-Producing Escherichia coli Sequence Types Carrying Quinolone and Aminoglycoside Resistance Genes Circulating in Companion and Domestic Farm Animals in Mwanza, Tanzania, Harbor Commonly Occurring Plasmids. *Front Microbiol* 2016;**7**:142. doi:10.3389/fmicb.2016.00142

204 Dramowski A, Cotton MF, Rabie H *et al.* Trends in paediatric bloodstream infections at a South African referral hospital. *BMC Pediatr* 2015;**15**:33. doi:10.1186/s12887-015-0354-3

205 Irenge LM, Kabego L, Kinunu FB *et al.* Antimicrobial resistance of bacteria isolated from patients with bloodstream infections at a tertiary care hospital in the Democratic Republic of the Congo. *S Afr Med J* 2015;**105**:752–5. doi:10.7196/SAMJnew.7937

206 Onanuga A, Omeje MC, Eboh DD. CARRIAGE OF MULTI-DRUG RESISTANT UROBACTERIA BY ASYMPTOMATIC PREGNANT WOMEN IN YENAGOA, BAYELSA STATE, NIGERIA. *Afr J Infect Dis* 2018;**12**:14–20. doi:10.21010/ajid.v12i2.3

207 Kateregga JN, Kantume R, Atuhaire C *et al.* Phenotypic expression and prevalence of ESBL-producing Enterobacteriaceae in samples collected from patients in various wards of Mulago Hospital, Uganda. *BMC Pharmacol Toxicol* 2015;**16**:14. doi:10.1186/s40360-015-0013-1

208 Opintan JA, Newman MJ, Arhin RE *et al.* Laboratory-based nationwide surveillance of antimicrobial resistance in Ghana. *Infect Drug Resist* 2015;**8**:379–89. doi:10.2147/idr.s88725

209 Pons MJ, Vubil D, Guiral E *et al.* Characterisation of extended-spectrum beta-lactamases

among Klebsiella pneumoniae isolates causing bacteraemia and urinary tract infection in Mozambique. *J Glob Antimicrob Resist* 2015;**3**:19–25. doi:10.1016/j.jgar.2015.01.004

210 Rafaï C, Frank T, Manirakiza A *et al.* Dissemination of IncF-type plasmids in multiresistant CTX-M-15-producing Enterobacteriaceae isolates from surgical-site infections in Bangui, Central African Republic. *BMC Microbiol* 2015;**15**. doi:10.1186/s12866-015-0348-1

211 Adeyankinnu FA, Motayo BO, Akinduti A *et al.* A Multicenter Study of Beta-Lactamase Resistant Escherichia coli and Klebsiella pneumoniae Reveals High Level Chromosome Mediated Extended Spectrum beta Lactamase Resistance in Ogun State, Nigeria. *Interdiscip Perspect Infect Dis* 2014;**2014**:819896. doi:10.1155/2014/819896

212 Irenge LM, Kabego L, Vandenberg O *et al.* Antimicrobial resistance in urinary isolates from inpatients and outpatients at a tertiary care hospital in South-Kivu Province (Democratic Republic of Congo). *BMC Res Notes* 2014;**7**:374. doi:10.1186/1756-0500-7-374

213 Scherbaum M, Kosters K, Murbeth RE *et al.* Incidence, pathogens and resistance patterns of nosocomial infections at a rural hospital in Gabon. *BMC Infect Dis* 2014;**14**:124. doi:10.1186/1471-2334-14-124

214 Yusuf I, Arzai AH, Haruna M *et al.* Detection of multi drug resistant bacteria in major hospitals in Kano, North-West, Nigeria. *Braz J Microbiol* 2014;**45**:791–8.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4204960/pdf/bjm-45-791.pdf

215 Alabi AS, Frielinghaus L, Kaba H *et al.* Retrospective analysis of antimicrobial resistance and bacterial spectrum of infection in Gabon, Central Africa. *BMC Infect Dis* 2013;**13**:455. doi:10.1186/1471-2334-13-455

216 Ibrahim ME, Bilal NE, Magzoub MA *et al.* Prevalence of Extended-spectrum beta-Lactamases-producing Escherichia coli from Hospitals in Khartoum State, Sudan. *Oman Med J* 2013;**28**:116–20. doi:10.5001/omj.2013.30

217 Seni J, Peirano G, Okon KO *et al.* The population structure of clinical extra-intestinal Escherichia coli in a teaching hospital from Nigeria. *Diagn Microbiol Infect Dis* 2018;**92**:46–9. doi:10.1016/j.diagmicrobio.2018.04.001

218 Obeng-Nkrumah N, Twum-Danso K, Krogfelt KA *et al.* High levels of extended-spectrum beta-lactamases in a major teaching hospital in Ghana: the need for regular monitoring and evaluation of antibiotic resistance. *Am J Trop Med Hyg* 2013;**89**:960–4. doi:10.4269/ajtmh.12-0642

219 Raji MA, Jamal W, Ojemhen O *et al.* Point-surveillance of antibiotic resistance in Enterobacteriaceae isolates from patients in a Lagos Teaching Hospital, Nigeria. *J Infect*

Public Health 2013;**6**:431–7. doi:10.1016/j.jiph.2013.05.002

220 Meeren BT van der, Chhaganlal KD, Pfeiffer A *et al.* Extremely high prevalence of multi-resistance among uropathogens from hospitalised children in Beira, Mozambique. *S Afr Med J* 2013;**103**:382–6. doi:10.7196/samj.5941

221 Idowu OJ, Onipede AO, Orimolade AE *et al.* Extended-spectrum Beta-lactamase Orthopedic Wound Infections in Nigeria. *J Glob Infect Dis* 2011;**3**:211–5. doi:10.4103/0974-777x.83524

222 Moyo SJ, Aboud S, Kasubi M *et al.* Antimicrobial resistance among producers and non-producers of extended spectrum beta-lactamases in urinary isolates at a tertiary Hospital in Tanzania. *BMC Res Notes* 2010;**3**:348. doi:10.1186/1756-0500-3-348

223 Bercion R, Mossoro-Kpinde D, Manirakiza A *et al.* Increasing prevalence of antimicrobial resistance among Enterobacteriaceae uropathogens in Bangui, Central African Republic. *J Infect Dev Ctries* 2009;**3**:187–90.https://jidc.org/index.php/journal/article/download/19759473/21

224 Mshana SE, Kamugisha E, Mirambo M *et al.* Prevalence of multiresistant gram-negative organisms in a tertiary hospital in Mwanza, Tanzania. *BMC Res Notes* 2009;**2**:49. doi:10.1186/1756-0500-2-49

225 Sire JM, Nabeth P, Perrier-Gros-Claude JD *et al.* Antimicrobial resistance in outpatient Escherichia coli urinary isolates in Dakar, Senegal. *J Infect Dev Ctries* 2007;**1**:263–8.https://jidc.org/index.php/journal/article/download/19734603/210

226 Blomberg B, Jureen R, Manji KP *et al.* High rate of fatal cases of pediatric septicemia caused by gram-negative bacteria with extended-spectrum beta-lactamases in Dar es Salaam, Tanzania. *J Clin Microbiol* 2005;**43**:745–9. doi:10.1128/jcm.43.2.745-749.2005

227 Gangoue-Pieboji J, Bedenic B, Koulla-Shiro S *et al.* Extended-spectrum-beta-lactamase-producing Enterobacteriaceae in Yaounde, Cameroon. *J Clin Microbiol* 2005;**43**:3273–7. doi:10.1128/jcm.43.7.3273-3277.2005

228 Zeynudin A, Pritsch M, Schubert S *et al.* Prevalence and antibiotic susceptibility pattern of CTX-M type extended-spectrum beta-lactamases among clinical isolates of gram-negative bacilli in Jimma, Ethiopia. *BMC Infect Dis* 2018;**18**:524. doi:10.1186/s12879-018-3436-7

229 Ndugulile F, Jureen R, Harthug S *et al.* Extended spectrum beta-lactamases among Gram-negative bacteria of nosocomial origin from an intensive care unit of a tertiary health facility in Tanzania. *BMC Infect Dis* 2005;**5**:86. doi:10.1186/1471-2334-5-86

230 Dromigny JA, Ndoye B, Macondo EA *et al.* Increasing prevalence of antimicrobial

resistance among Enterobacteriaceae uropathogens in Dakar, Senegal: A multicenter study. *Diagn Microbiol Infect Dis* 2003;**47**:595–600. doi:10.1016/S0732-8893(03)00155-X

231 Dromigny JA, Nabeth P, Perrier Gros Claude JD. Distribution and susceptibility of bacterial urinary tract infections in Dakar, Senegal. *Int J Antimicrob Agents* 2002;**20**:339–47.https://www.ijaaonline.com/article/S0924-8579(02)00196-6/fulltext

232 Ampaire L, Nduhura E, Wewedru I. Phenotypic prevalence of extended spectrum beta-lactamases among enterobacteriaceae isolated at Mulago National Referral Hospital: Uganda. *BMC Res Notes* 2017;**10**:448. doi:10.1186/s13104-017-2786-3

233 Andrew B, Kagirita A, Bazira J. Prevalence of Extended-Spectrum Beta-Lactamases-Producing Microorganisms in Patients Admitted at KRRH, Southwestern Uganda. *Int J Microbiol* 2017;**2017**:3183076. doi:10.1155/2017/3183076

234 Archary M, Adler H, La Russa P *et al.* Bacterial infections in HIV-infected children admitted with severe acute malnutrition in Durban, South Africa. *Paediatr Int Child Health* 2017;**37**:6–13. doi:10.1080/20469047.2016.1198561

235 Henson SP, Boinett CJ, Ellington MJ *et al.* Molecular epidemiology of Klebsiella pneumoniae invasive infections over a decade at Kilifi County Hospital in Kenya. *Int J Med Microbiol* 2017;**307**:422–9. doi:10.1016/j.ijmm.2017.07.006

236 Chirindze LM, Zimba TF, Sekyere JO *et al.* Faecal colonization of E. coli and Klebsiella spp. producing extended-spectrum beta-lactamases and plasmid-mediated AmpC in Mozambican university students. *BMC Infect Dis* 2018;**18**:244. doi:10.1186/s12879-018-3154-1

237 Founou RC, Founou LL, Essack SY. Extended spectrum beta-lactamase mediated resistance in carriage and clinical gram-negative ESKAPE bacteria: a comparative study between a district and tertiary hospital in South Africa. *Antimicrob Resist Infect Control* 2018;**7**:134. doi:10.1186/s13756-018-0423-0

238 Magwenzi MT, Gudza-Mugabe M, Mujuru HA *et al.* Carriage of antibiotic-resistant Enterobacteriaceae in hospitalised children in tertiary hospitals in Harare, Zimbabwe. *Antimicrob Resist Infect Control* 2017;**6**:10. doi:10.1186/s13756-016-0155-y

239 Moremi N, Claus H, Vogel U *et al.* Faecal carriage of CTX-M extended-spectrum beta-lactamase-producing Enterobacteriaceae among street children dwelling in Mwanza city, Tanzania. *PLoS One* 2017;**12**:e0184592. doi:10.1371/journal.pone.0184592

240 Wilmore SMS, Kranzer K, Williams A *et al.* Carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae in HIV-infected children in Zimbabwe. *J Med Microbiol* 2017;**66**:609–15. doi:10.1099/jmm.0.000474

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

242 Desta K, Woldeamanuel Y, Azazh A *et al.* High Gastrointestinal Colonization Rate with Extended-Spectrum beta-Lactamase-Producing Enterobacteriaceae in Hospitalized Patients: Emergence of Carbapenemase-Producing K. pneumoniae in Ethiopia. *PLoS One* 2016;**11**:e0161685. doi:10.1371/journal.pone.0161685

243 Djuikoue IC, Woerther PL, Toukam M *et al.* Intestinal carriage of Extended Spectrum Beta-Lactamase producing E. coli in women with urinary tract infections, Cameroon. *J Infect Dev Ctries* 2016;**10**:1135–9. doi:10.3855/jidc.7616

244 Mshana SE, Falgenhauer L, Mirambo MM *et al.* Predictors of blaCTX-M-15 in varieties of Escherichia coli genotypes from humans in community settings in Mwanza, Tanzania. *BMC Infect Dis* 2016;**16**:187. doi:10.1186/s12879-016-1527-x

245 Ribeiro TG, Novais Â, Peixe L *et al.* Atypical epidemiology of CTX-M-15 among Enterobacteriaceae from a high diversity of non-clinical niches in Angola. *Journal of Antimicrobial Chemotherapy* 2016;**71**:1169–73. doi:10.1093/jac/dkv489

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

247 Chereau F, Herindrainy P, Garin B *et al.* Colonization of extended-spectrum-beta-lactamase- and NDM-1-producing Enterobacteriaceae among pregnant women in the community in a low-income country: a potential reservoir for transmission of multiresistant Enterobacteriaceae to neonates. *Antimicrob Agents Chemother* 2015;**59**:3652–5. doi:10.1128/aac.00029-15

248 Herindrainy P, Rabenandrasana MAN, Andrianirina ZZ *et al.* Acquisition of extended spectrum beta-lactamase-producing enterobacteriaceae in neonates: A community based cohort in Madagascar. *PLoS One* 2018;**13**:e0193325. doi:10.1371/journal.pone.0193325

249 Micheel V, Hogan B, Rakotoarivelo RA *et al.* Identification of nasal colonization with beta-lactamase-producing Enterobacteriaceae in patients, health care workers and students in Madagascar. *Eur J Microbiol Immunol (Bp)* 2015;**5**:116–25. doi:10.1556/eujmi-d-15-00001

250 Nelson E, Kayega J, Seni J *et al.* Evaluation of existence and transmission of extended spectrum beta lactamase producing bacteria from post-delivery women to neonates at Bugando Medical Center, Mwanza-Tanzania. *BMC Res Notes* 2014;**7**:279. doi:10.1186/1756-0500-7-279

251 Lonchel CM, Melin P, Gangoue-Pieboji J *et al.* Extended-spectrum beta-lactamase-producing Enterobacteriaceae in Cameroonian hospitals. *Eur J Clin Microbiol Infect Dis* 2013;**32**:79–87. doi:10.1007/s10096-012-1717-4

252 Magoue CL, Melin P, Gangoue-Pieboji J *et al.* Prevalence and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Ngaoundere, Cameroon. *Clin Microbiol Infect* 2013;**19**:E416–20. doi:10.1111/1469-0691.12239

253 Schaumburg F, Alabi A, Kokou C *et al.* High burden of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Gabon. *J Antimicrob Chemother* 2013;**68**:2140–3. doi:10.1093/jac/dkt164

254 Albrechtova K, Dolejska M, Cizek A *et al.* Dogs of nomadic pastoralists in northern Kenya are reservoirs of plasmid-mediated cephalosporin- and quinolone-resistant Escherichia coli, including pandemic clone B2-O25-ST131. *Antimicrob Agents Chemother* 2012;**56**:4013–7. doi:10.1128/aac.05859-11

255 Isendahl J, Turlej-Rogacka A, Manjuba C *et al.* Fecal carriage of ESBL-producing E. coli and K. pneumoniae in children in Guinea-Bissau: a hospital-based cross-sectional study. *PLoS One* 2012;**7**:e51981. doi:10.1371/journal.pone.0051981

256 Lonchel CM, Meex C, Gangoué-Piéboji J *et al.* Proportion of extended-spectrum ß-lactamase-producing Enterobacteriaceae in community setting in Ngaoundere, Cameroon. *BMC Infect Dis* 2012;**12**. doi:10.1186/1471-2334-12-53

257 Herindrainy P, Randrianirina F, Ratovoson R *et al.* Rectal carriage of extended-spectrum beta-lactamase-producing gram-negative bacilli in community settings in Madagascar. *PLoS One* 2011;**6**:e22738. doi:10.1371/journal.pone.0022738

258 Woerther PL, Angebault C, Jacquier H *et al.* Massive increase, spread, and exchange of extended spectrum beta-lactamase-encoding genes among intestinal Enterobacteriaceae in hospitalized children with severe acute malnutrition in Niger. *Clin Infect Dis* 2011;**53**:677–85. doi:10.1093/cid/cir522

259 Katakweba AAS, Muhairwa AP, Lupindu AM *et al.* First Report on a Randomized Investigation of Antimicrobial Resistance in Fecal Indicator Bacteria from Livestock, Poultry, and Humans in Tanzania. *Microbial Drug Resistance* 2018;**24**:260–8. doi:10.1089/mdr.2016.0297

260 Andriatahina T, Randrianirina F, Hariniana ER *et al.* High prevalence of fecal carriage of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in a pediatric unit in Madagascar. *BMC Infect Dis* 2010;**10**:204. doi:10.1186/1471-2334-10-204

261 Ruppe E, Woerther PL, Diop A *et al.* Carriage of CTX-M-15-producing Escherichia coli

isolates among children living in a remote village in Senegal. *Antimicrob Agents Chemother* 2009;**53**:3135–7. doi:10.1128/aac.00139-09

262 Tande D, Jallot N, Bougoudogo F *et al.* Extended-spectrum beta-lactamase-producing Enterobacteriaceae in a Malian orphanage. *Emerg Infect Dis* 2009;**15**:472–4. doi:10.3201/eid1503.071637

263 Marando R, Seni J, Mirambo MM *et al.* Predictors of the extended-spectrum-beta lactamases producing Enterobacteriaceae neonatal sepsis at a tertiary hospital, Tanzania. *Int J Med Microbiol* 2018;**308**:803–11. doi:10.1016/j.ijmm.2018.06.012

264 Moremi N, Claus H, Rutta L *et al.* High carriage rate of extended-spectrum beta-lactamase-producing Enterobacteriaceae among patients admitted for surgery in Tanzanian hospitals with a low rate of endogenous surgical site infections. *J Hosp Infect* 2018;**100**:47–53. doi:10.1016/j.jhin.2018.05.017

265 Nikiema Pessinaba C, Landoh DE, Dossim S *et al.* Screening for extended-spectrum beta-lactamase-producing Enterobacteriaceae intestinal carriage among children aged under five in Lome, Togo. *Med Mal Infect* 2018;**48**:551–4. doi:10.1016/j.medmal.2018.07.004

266 Sanneh B, Kebbeh A, Jallow HS *et al.* Prevalence and risk factors for faecal carriage of Extended Spectrum beta-lactamase producing Enterobacteriaceae among food handlers in lower basic schools in West Coast Region of The Gambia. *PLoS One* 2018;**13**:e0200894. doi:10.1371/journal.pone.0200894

267 Stanley IJ, Kajumbula H, Bazira J *et al.* Multidrug resistance among Escherichia coli and Klebsiella pneumoniae carried in the gut of out-patients from pastoralist communities of Kasese district, Uganda. *PLoS One* 2018;**13**. doi:10.1371/journal.pone.0200093

268 Kurz MS, Bayingana C, Ndoli JM *et al.* Intense pre-admission carriage and further acquisition of ESBL-producing Enterobacteriaceae among patients and their caregivers in a tertiary hospital in Rwanda. *Trop Med Int Health* 2017;**22**:210–20. doi:10.1111/tmi.12824

269 Gray KJ, Wilson LK, Phiri A *et al.* Identification and characterization of ceftriaxone resistance and extended-spectrum β-lactamases in Malawian bacteraemic Enterobacteriaceae. *Journal of Antimicrobial Chemotherapy* 2006;**57**:661–5. doi:10.1093/jac/dkl037

270 Musicha P, Feasey NA, Cain AK *et al.* Genomic landscape of extended-spectrum β-lactamase resistance in Escherichia coli from an urban African setting. *Journal of Antimicrobial Chemotherapy* 2017;**72**:1602–9. doi:10.1093/jac/dkx058

271 Musicha P, Msefula CL, Mather AE *et al.* Genomic analysis of Klebsiella pneumoniae isolates from Malawi reveals acquisition of multiple ESBL determinants across diverse lineages.

Journal of Antimicrobial Chemotherapy 2019;**74**:1223–32. doi:10.1093/jac/dkz032