

Developing an Antimicrobial Strategy for Sepsis in Malawi

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Preface

Joe's thesis

Chapter 1

Introduction

1.1 Chapter Overview

The syndrome of sepsis is an ancient one; from Hippocrates to Galen and Semmelweis, the potentially serious consequences of infection have long been recognized. Modern definitions of sepsis conceptualise it as a syndrome of life threatening organ dysfunction due to a deleterious and dysregulated host response to infection, but despite increased understanding of its pathogenesis, mortality from sepsis remains high. Progress has been made in improving sepsis mortality in high income settings through timely application of basic care: 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, and increasing evidence suggests that exporting high-income setting sepsis protocols to sSA has the potential to do harm. 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. The effects of this at an individual level are unknown, but 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. 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 - a first and last line antibiotic - 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 will argue that sustainable antimicrobial strategies for sepsis in sSA can not only consider mortality; the unintended consequences in terms of antimicrobial resistance (AMR) acquisition in a setting where empiric management of infection is the norm must also be considered, and mitigated against where possible. In this chapter, I will review, firstly, the definitions, epidemiology, aetiology and management of sepsis, with a focus on aetiology and antimicrobial treatment; and secondly, 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 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). 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[1–8] and three intervention studies (two RCTs[9,10] and one before-after intervention[11]) 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 hits and number of included studies after full text review: nine studies provided data on Leptospirosis[12–20], seven on Brucellosis[21–27], seven on Q-fever[19,23,28–31] , six on Rickettsioses[19,28,32–35], eighteen on Dengue[13,15,19,20,28,34,36–47], thirteen on Chikungunya[15,20,34,37,40,42,44–50], three on Zika [43–45], two on Histoplasmosis[51,52] 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
Leprosirosis	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 Defining sepsis

Sepsis is a heterogenous syndrome, with no diagnostic gold standard. In 1991 the first modern sepsis diagnostic criteria were defined in a consensus conference of key opinion makers[53]

(Table 1.2). 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 nonspecific for the diagnosis of sepsis led to an expansion of the diagnostic criteria in 2001[54] 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[55].

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, Appendix), an organ-dysfunction score already in use in high income settings, and shown to be associated with mortality[56] 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 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)[57].

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. 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[58].

1.2.3 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[59] or be difficult to access[60], 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[6,61–64] 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.

1.2.4 Sepsis epidemiology in sub-Saharan Africa

1.2.4.1 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[65], 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[66]. 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.

1.2.4.2 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[67,68]. In the United States, male sex and black ethnicity (vs white) and poverty are associated with increased sepsis incidence and severity[69].

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 1.3;

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/ μ L. 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[67,70,71]. The need for data from sSA to guide sepsis treatment protocols, rather than extrapolating from the high-income setting sepsis population, is clear.

1.2.4.3 Outcomes

Summary outcomes for sepsis and severe sepsis in sSA from the identified studies are presented in Figure 1.1 below. Summary statistics of 28 or 30-day 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 in R. Exact binomial 95% mortality confidence intervals were used throughout.

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%)[66], 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%[57,72,73]. Most recent (largely post-2005) estimates of 30-day mortality from severe sepsis range from 18-29%[65,66,71,74,75]. It seems likely therefore, that both sepsis and severe sepsis 30-day mortality is considerably higher in sSA than in high-income settings. The reasons for this are not clear, but are likely to be multifactorial; resource limitation is likely to play a part but the HIV epidemic in sSA, differing pathogen

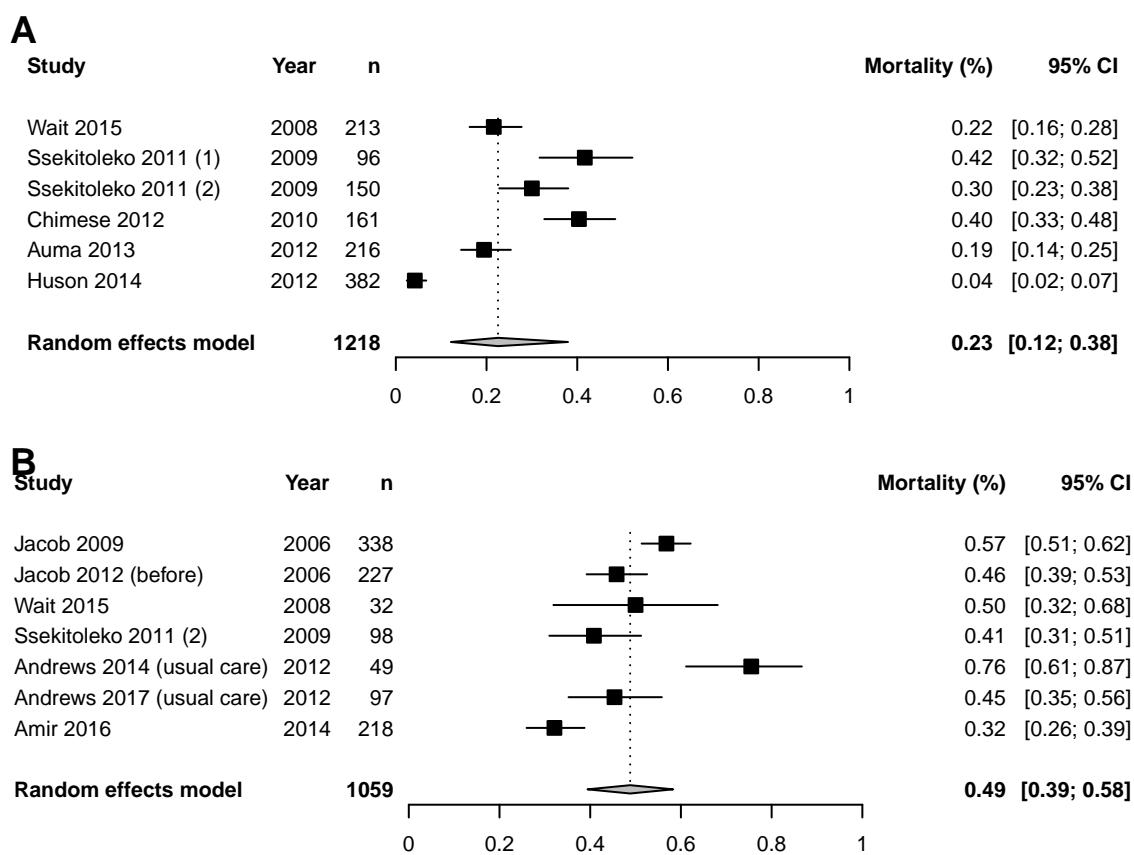


Figure 1.1: Pooled sepsis (A, top) and severe sepsis (B, bottom) inpatient mortality in sSA

burden and lack of data and evidence based guidelines to inform optimal management in sSA may also play a role.

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[76]. Both short and long term morbidity is formidable also, though, once again, data from low income settings including sSA are absent. Cohort studies with no comparator group may not identify morbidity that is sepsis-specific (rather morbidity that is related to critical illness) but new, long-lasting reduction in physical and cognitive function with associated functional impairment have been identified in matched cohort studies in sepsis survivors[77,78]. Health-related quality of life in sepsis survivors in high-income settings have been found to be persistently below population norms[76]. Increased incidence of cardiovascular disease, renal failure and further episodes of infection are seen following a hospital discharge for sepsis[79–81]. Long term sepsis outcomes in sSA are unknown.

1.2.5 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 1.4 and 1.5 below. 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 is almost exclusively HIV-associated. The importance of bacteraemic tuberculosis as a cause of sepsis is further examined in an individual patient data meta analysis in chapter 3. With the exception of one study, malaria was less common than BSI, highlighting the importance of non-malarial fever in sSA as malaria control efforts reduce the burden of malaria.

1.2.5.1 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[82]. 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 Chikungunya[83].

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 below 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[84] 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 *Leptospira 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[85] 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[86] 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[87]. 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%]).

1.2.5.2 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[88]. It is likely therefore to contribute significantly to aetiology of sepsis; of the 11 identified sSA sepsis cohorts, three[4,5,9] provide data on suspected site of infection, and CNS infection accounts for 14-31% of the total, of which cryptococcal disease is likely to be responsible for a large proportion. One study² performed CSF examination on 41/213 patients for suspected meningoencephalitis. Of these, 3/41 cultured *C. neoformans*.

No study has attempted to define the burden of PCP in sepsis in sSA, though a 2016 systematic review[89] addressed the prevalence and attributable mortality of PCP. Searches were limited to post-1995; 48 studies were identified comprising 6884 individuals from 18 countries, with a varying patient population including inpatients and outpatients with respiratory presentation

or clinical or radiological community acquired pneumonia, often sputum smear negative for TB, and some autopsy studies. A number of diagnostic tests including bronchoscopy and bronchoalveolar lavage were carried out. Many of the inpatient cohorts would include patients with sepsis; the pooled prevalence of PCP in inpatients ($n = 2593$, 23 studies) was 22% (90% CI 17 – 27%) in random effect meta-analysis. Clearly there are significant difficulties with obtaining lower respiratory tract specimens in unwell hypoxic, shocked or obtunded patients; newer serologic tests (1,3, beta-d glucan) which have reasonable diagnostic characteristics for PCP in high-income settings[90] and may have a role to play, but no study in sSA has attempted to use or validate this assay in any condition.

Data examining the role of Histoplasmosis as a cause of fever or sepsis in sSA are sparse. A 2015 systematic review[83] identified only one study up to 2013 which Histoplasma urine antigen testing in 628 febrile adults and children in Tanzania and acute serum testing on a subset of 200, finding 9/628 (1%) probable cases, 6/9 of whom were HIV infected. Since then, two studies have addressed histoplasma prevalence in varying conditions: the first, in Uganda, enrolled HIV-infected patients with suspected meningitis[51] and found 0/151 patients had detectable IgM to *Histoplasma capsulatum* and no Histoplasma antigen was detected in serum ($n = 57$), urine ($n = 37$) or CSF ($n=63$). The second study in Cameroon[52] recruited HIV infected patients with $CD4 < 200 \text{ cells}/\mu\text{L}$, chronic cough and Histoplasmosis like skin manifestations. Histopathologic examination and culture found Histoplasmosis in 7/56 (13%) of patients over 3 years.

1.2.6 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[91] are shown in Table 1.6 below.

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[92], endorsed by a number of national and international sepsis organisations, and supplements in 2016-17 covering general supportive care[93], infection management[94], management of severe malaria and severe dengue[92] and haemodynamic assessment and support[95] in sepsis in low-resource settings. The major recommendations of this guidance are consolidated in Table 1.7 below.

The World Health Organisation (WHO) in 2011 published the integrated management of adolescent and adult illness (IMAI) guidance[87], 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.

1.2.6.1 Early goal directed therapy

In 2001 a pivotal single centre study in the United States of 263 patients with severe sepsis or septic shock[96] 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[97], ARISE in Australasia[98] and ProMISe[99] 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[71]. 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 in 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.

1.2.6.2 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[100]. 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[101], 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[70] 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[1], 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[11], 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[102] 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[103]: 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.

1.2.6.3 Evidence to guide 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: one multicentre study of 2796 adults with severe sepsis[104] found no propensity adjusted difference in in-hospital mortality for patients with shock or elevated lactate whether they received fluid bolus within the first 6hr following diagnosis (aOR 1.01 [95% CI 0.73 – 1.39]); the New York study of 49,331 septic adults described above[70] found no association between time to completion of fluid bolus and mortality (aOR 1.01 per hour [95% CI 0.99 – 1.02]). 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[@[[70]; Boyd2011; Vincent2006].

In contrast, several studies contradict these findings; a retrospective single centre of 594 adults with severe sepsis or septic shock[105] found improved mortality in patients who had a higher proportion of 6-hour fluid administered in the first 3hr, when adjusted for total volume of fluid administered over 6hr (aOR 0.34 [95% CI 0.15 – 0.75]); a larger retrospective multicentre study of 11,182 patients with sepsis and hypotension[106] found an independent mortality benefit for early intravenous crystalloid administration, with fluid administration within 30mins having the largest effect (aOR 0.74 [95% CI 0.62 – 0.87] versus > 120mins). A prospective study of 1866 patients from the same authors[107] had similar findings (aOR 0.63 [95% CI 0.46-0.86]).

It may be that heterogeneity in response to fluids plays a role in these conflicting findings; a retrospective multicentre cohort analysis of 3686 patients[108] found that 64% were “fluid responders” – that is, they had a sustained blood pressure response to initial fluid resuscitation without need for vasopressors. Heart failure, hypothermia, altered gas exchange, initial lactate > 4.0mmol/L, coagulopathy and immune compromise (including HIV/AIDS) were associated with fluid nonresponse, as was fluid initiation greater than 2 hours after sepsis diagnosis. Mortality was 15% greater (95% CI 10-18%) in fluid nonresponders.

In sSA, there is increasing evidence that liberal intravenous fluid administration to septic patients causes harm. The landmark FEAST trial[109] 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[110] 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; the first is the before-after intervention study in septic shock patients carried out in Uganda and described above[11]. 426 patients were included in the intervention with 245 in the usual care group; the intervention consisted of clinical-officer delivered protocolised care over the first 6 hours of hospital admission. The intervention increased fluid administration over 6 hours (3.0L vs 0.5L, $p < 0.001$) and 24 hours (3.5L vs 1.0L, $p < 0.001$), and more patients received fluid within 1 hour (97% vs 55%, $p < 0.001$). The study 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] for 1.0 – 2.5 L vs < 1.0L) though with the absence of any further dose-response effect above 1L. As stated above, the before-after study design means that this result should be interpreted with caution.

Two randomised controlled trials of protocolised early sepsis care in adults have been carried out at a single centre in Zambia, with a focus on fluid. The first[9] recruited patients with severe sepsis with organ dysfunction criteria including respiratory rate > 40/min. Patients were randomised to usual care or an intervention protocol consisting of a 2L bolus of crystalloid (lactated Ringer's or 0.9% saline) over 1 hour and then, if the jugular venous pressure (JVP) was below 3cm, a further 2L over 4 hours. Fluids were stopped if worsening respiratory signs or symptoms developed. If MAP was below 65mmHg after 2L of fluid, a dopamine infusion was started. Blood was transfused if Hb was < 7g/dL. This trial was stopped early (after recruitment of 109 patients) as it was felt that participants with baseline respiratory compromise (RR > 40 or oxygen saturation < 90%) 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 same intervention was then used at the same centre in a similar trial[Andrews2017], this time recruiting patients with two SIRS criteria and hypotension (SBP < 90mmHg or MAP < 65mmHg), but excluding patients with baseline respiratory compromise(RR > 40/min or oxygen saturation < 90%) and randomising them 1:1 to the intervention protocol. 209 patients were recruited and patients in the intervention group ($n = 106$) at 6 hours received more fluid (median 3.5L vs 2.0L, $p <0.001$) with more vasopressor use (12% vs 2%, $p = 0.01$), but similar proportions of blood transfusion (16% vs 12%, $p = 0.48$). Lactate change by 6 hours was greater in the intervention group (median -1.2 vs -0.5 mmol/L, $p = 0.02$), but so too was in hospital mortality (48% vs 33%, $p = 0.03$). The reasons for this 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

1.3.1 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 (((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)).

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. 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[111–163] providing data on invasive infection and 32 [164–196] 167–199 on carriage. Details of these studies are given below. A broad non-systematic review of the literature was also undertaken to place these studies in context and provide a background understanding of the classification and global epidemiology of ESBL-E, using the same literature databases.

1.3.2 Introduction: definition and classification of ESBL-E

Beta-lactamases are enzymes that hydrolyse the active beta lactam ring in beta lactam antimicrobials. Though no standardised definition of ESBL exists, they are usually defined as enzymes which confer resistance via hydrolysis to penicillins, cephalosporins of the first, second or third generation (excluding cephemycins), aztreonam, but not carbapenems, and are inhibited by beta-lactamase inhibitors such as clavulanic acid[197].

Two classification schemes are usually used for ESBL: the molecular (or structural) classification of Ambler[198], or the Bush-Jacoby-Medeiros functional classification[199] (Table 1.8). Molecular classification is straightforward and depends on protein homology; class A, C and D enzymes are serine beta-lactamases and class B are metallo-beta 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 beta-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 beta lactamases that are largely inhibited by clavulanic acid and belong to Ambler class A or C; and class 3 are the metallo-beta-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 14. The vast majority of clinically relevant ESBLs (and all of those defined as above) belong to Ambler class A, functional class 2be.

Table 1.8: ESBL classification. Adapted from Bush (2010)

Bush Jacoby group	Molecular class	Distinctive substrates	Inhibited by		Defining hydrolysis spectrum characteristics	Representative enzymes
			BLI	EDTA		
1	C	Cephalosporins	No	No	cephalosporins > benpen, hydrolyzes cephemycins	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, monobac- tams	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, monobac- tams	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	OXA-11, OXA-15
2df	D	Carbapenems	Variable	No	oxyimino-beta-lactams cloxacillin or oxacillin and carbapenems	OXA-23, OXA-48
2e	A	Extended- spectrum cephalosporins	Yes	No	Inhibited by clavulanic acid but not aztreonam	CepA
2f	A	Carbapenems	Variable	No	carbapenems, oxyimino-beta-lactams, cephemycins	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,

1.3.3 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 beta-lactamase genes, many of them ESBL. However, there are 3 families 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[200]; like TEM and SHV, OXA beta-lactamases are not always extended-spectrum.

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

Beta-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[201]. These beta-lactamases are often chromosomally located; the first plasmid-mediated narrow-spectrum beta-lactamase, TEM-1 -named for the patient, Temoneira, from whose blood it was first isolated – was found in Athens in the 1960s[202]. It rapidly disseminated globally and is thought to be responsible for a high proportion of ampicillin resistance in *E. coli*, for example[200]. This worldwide spread spurred the development and use of beta-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, carried on a pBP60 plasmid and enzymes of this sort were named ESBLs[203,204].

This first ESBL enzyme was found to be similar to an existing plasmid-borne 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 chromosomal *K. pneumoniae* narrow spectrum beta lactamase which was liberated onto a plasmid[205]. The point mutations in SHV-1 conferred the ESBL phenotype, and this enzyme was named SHV-2. This pattern - mutation of a narrow spectrum beta-lactamase to produce an ESBL phenotype - also occurred in TEM, and the first ESBL TEM was described in France in 1989[206] and named TEM-3. Many TEM and SHV variants were subsequently described[207]. However, in this early stage of the epidemic, ESBL enzymes

were largely nosocomial, and often associated with *Klebsiella spp.*[208].

1.3.3.2 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[209], aided by mobile genetic elements; second, *E. coli* joining *Klebsiella spp.* as a major ESBL host[210], and the emergence of so-called high risk bacterial clones; and third, the spread of ESBL-E into the community[211]. CTX-M-1 was first identified and named in Germany in 1989[212] and many variants were subsequently identified, largely in *E. coli* and *K. pneumoniae*, from isolates all over the world[213]. CTX-M genes are clustered by homology into 5 groups (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.*[209]

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 locations[[210]; Bevan2017]. 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[211], 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 (see carriage, below).

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[[214]; Lai2011] and economic status; GDP per capita has been found to correlate inversely at a country level with third-generation cephalosporin resistance rates[215]. Data from sSA have historically been lacking and are systematically reviewed below.

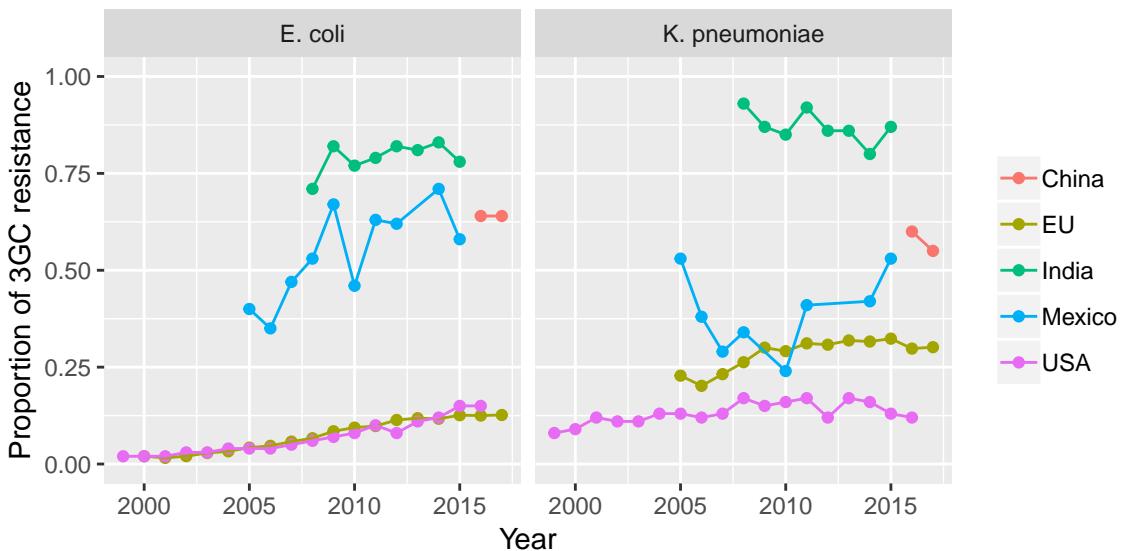


Figure 1.2: Prevalence of third generation cephalosporin resistance in representative high income (EU, USA) and middle income (China, Mexico, India) areas in invasive *E. coli* (left panel) and *K. pneumoniae* (right panel) isolates. Data for EU are from ECDC surveillance atlas (<https://ecdc.europa.eu/en/antimicrobial-resistance/surveillance-and-disease-data/data-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.

1.3.3.3 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[216], and whole genome sequencing has confirmed that invasive isolates are often closely related to prior gut carriage isolates[217]. 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[218] and healthy children in Poland[219] was first described in 2001, and subsequently has been identified worldwide when sought[220], 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[221], 4.5% in the Netherlands in 2012[222] and 4.7% in Sweden in 2012/13[223] and 3.7% in Spain in 2003[224], significantly lower than community carriage prevalence of 50.9% seen in China in 2009[225] or 33.8% in India in 2011-2013[226].

Risk factors for colonisation have been identified in many studies and antimicrobial exposure[227,228] and healthcare facility exposure[[226]; Luvsansharav2012] (including long term care facilities[229]) are consistently identified as such. Colonisation of a household member has also been identified as a risk factor[[230]; Rodriguez-Bano2008], suggesting significant within-household spread. Antacid use has been associated with ESBL-E colonisation[227] as has exposure to farming[@[222]. In low prevalence areas, travel to high prevalence areas is a risk factor[221,223,227,228,231].

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[232]. French and German studies found a median duration of carriage of 4.3[233] and 12.5[234] 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[235]; 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 CTX-M-27 or 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[231,236]. 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.

1.3.3.4 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 CTX-M from *Kluyvera spp.* is thought to have been mediated by capture of transposable insertion sequences; the insertion sequence IS_{Ecp1} has been experimentally demonstrated to mobilise the CTX-M precursor from *Kluyvera ascorbata*[237] and IS_{Ecp1} is most consistently associated with CTX-M genes but IS26, ISCR1 and IS10 have also persistently been described upstream from CTX-M genes, suggesting multiple mobilisation events[238]. There is also an association between particular pairs of CTX-M gene clusters and insertion sequences, consistent with a hypothesis of multiple mobilisation events[239]. These insertion sequences provide two roles: they encode a transposase enabling gene mobilisation but act as a strong promotor of CTX-M, without which phenotypic cephalosporin resistance is absent or reduced[240].

After mobilisation from the *Kluyvera* genome, the CTX-M genes were integrated onto a plasmid backbone, a process which is likely ongoing as a substantial number of diverse CTX-M carrying plasmids have been described: there is, however, an association between CTX-M genotype and plasmid incompatibility group. The successful CTX-M 15 gene is very strongly associated with the narrow host-range IncF plasmid group, for example, which are restricted to Enterobacteriaceae[[240]; Carattoli2009]. Identical CTX-M containing plasmids have been found across diverse geographical regions and have been termed “epidemic plasmids”[238]

though the mechanism of persistence of these plasmids within a bacterial population remains unclear.

In addition to frequently co-occurring CTX-M genes, transposable elements and plasmids, some clonal groups of *E. coli* and *K. pneumoniae* are both globally successful and associated with particular 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 CTX-M-15[241]. First described in 2008, *E. coli* ST131 is thought to be responsible for around 80% of extra-intestinal ESBL *E. coli* infection[242]. 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[243,244]. These events may have contributed to the global success of ST131, but the precise mechanism of its apparent fitness advantage remains unknown.

1.3.4 Epidemiology of ESBL-E in sub-Saharan Africa

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 in sSA. These are considered in turn below.

1.3.4.1 Invasive ESBL-E infection

Table xx in appendix xx 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 ??A shows a map 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 ??B and ??C), 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.

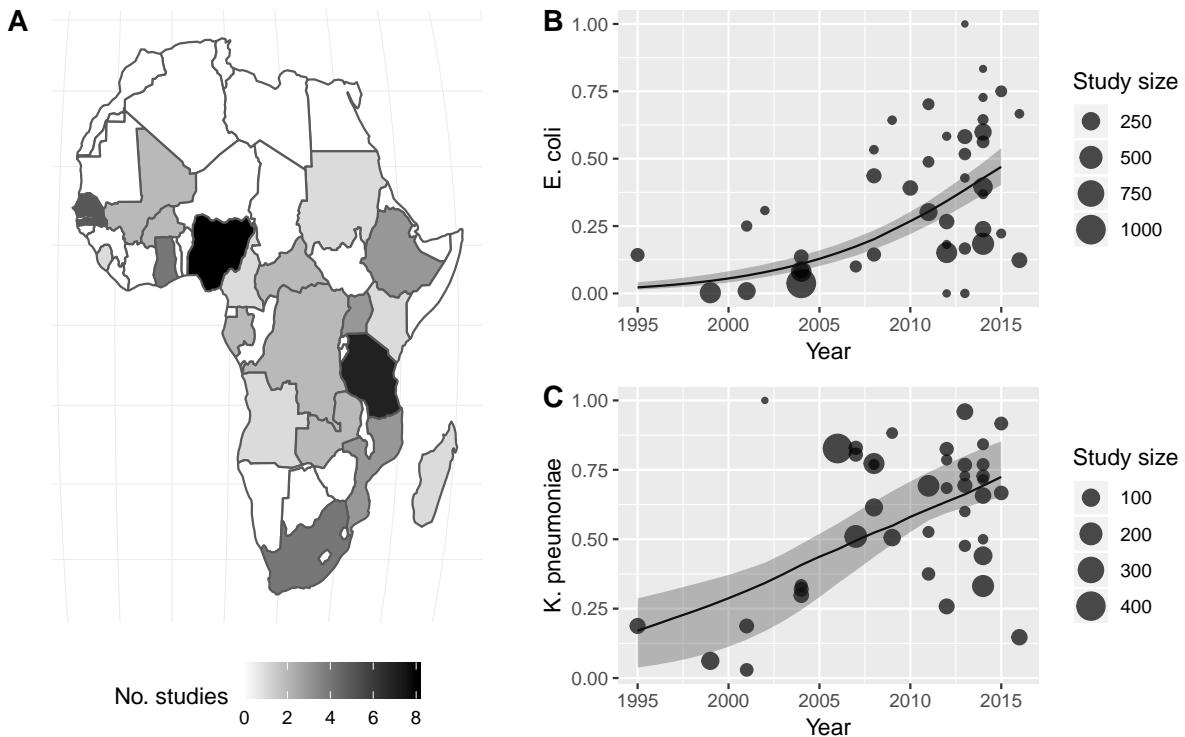


Figure 1.3: Invasive ESBL-E in sSA. A: Available studies by country. B and C: meta regression of proportion of invasive *E. coli* and *K. pneumoniae* respectively as a function of time. In both cases time is statistically significantly associated with proportion of ESBL ($p < 0.001$ on likelihood ratio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

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 SHV-12 enzyme predominant[155]; in Dakar, Senegal, 6/97 *K. pneumoniae* isolates from community acquired urinary tract infections in 1999-2000 were found to be ESBL producers[158].

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)[115,117–122,124,127,128,130,132,137,140,154,163]. 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 binomial-normal random effects meta analysis was

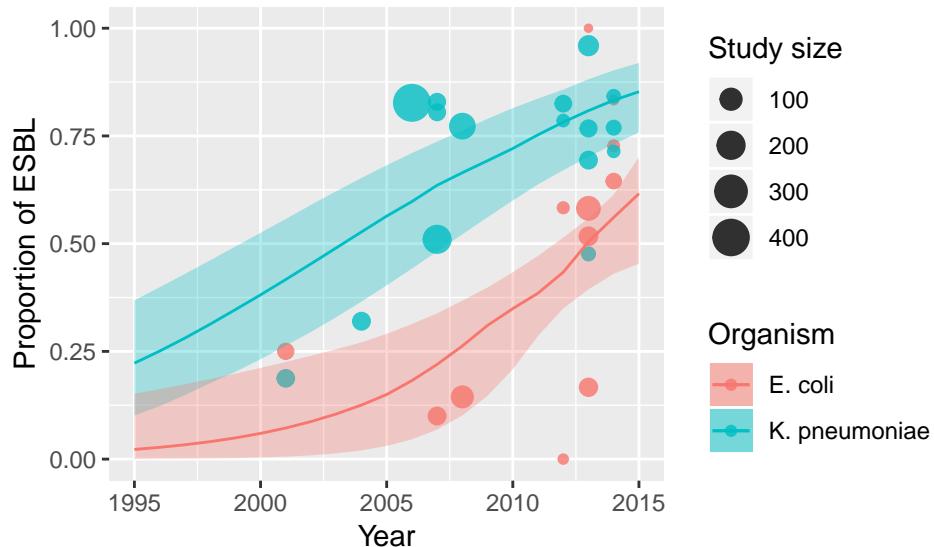


Figure 1.4: Meta regression of proportion of ESBL producing *E. coli* and *K. pneumoniae* amongst invasive isolate in SSA from studies carrying out blood culture, as a function of time. Includes 1242 *K. pneumoniae* and 489 *E. coli* isolates. 95% CI generated from 1000 bootstrap replicates from fitted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

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[163] ($n=1$) or by PCR[116,119,120,122,129,137,138,154,157] ($n=9$) for 821 *E. coli* and 791 *K. pneumoniae* isolates (Figure 1.5). CTX-M enzymes were the most commonly occurring ESBL genes, and the majority of these were CTX-M-15 in both organisms. OXA, TEM and 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, SHV-1 and TEM-1 are narrow spectrum beta lactamase enzymes, which were commonly identified in these studies, though only a handful of isolates had characterisation of SHV enzymes beyond identification of the SHV group. All the identified OXA genes were narrow spectrum beta lactamases (OXA-1). These data suggest that the genomic landscape of invasive ESBL-E in sSA is dominated by CTX-M, and 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 Enterobacteriaceae

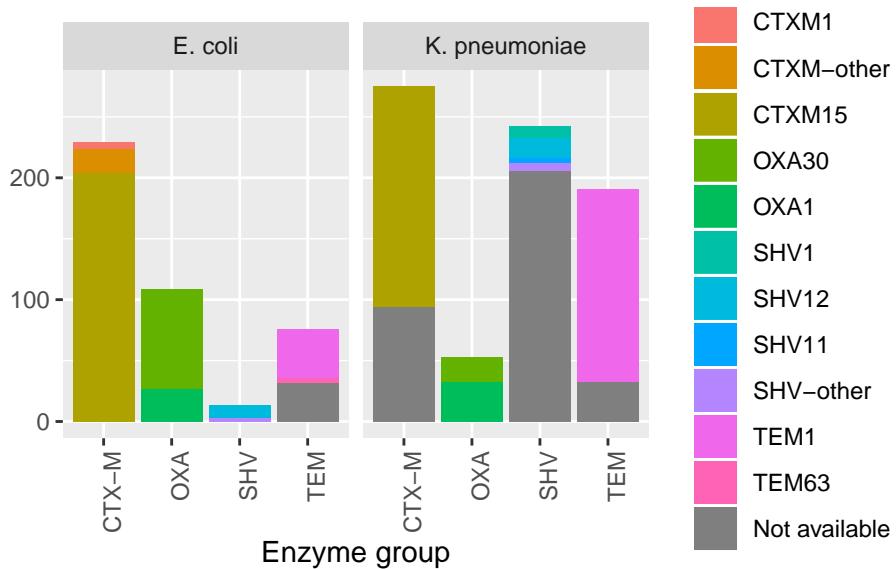


Figure 1.5: Distribution of beta-lactamase genes in invasive ESBL producing *E. coli* (n= 821) and *K. pneumoniae* (n = 791) from 10 studies

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. 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[245]. 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[246], though this study did not carry out confirmatory ESBL testing. Finally, a retrospective whole-genome sequencing study which selected 94 diverse (largely invasive) clinical *E. coli* isolates from Blantyre from 1996-2014 found that 21/94 isolates carried an ESBL gene, with CTX-M predominating (20/21)[247].

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

Table xx in appendix xx 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 (Figure A). 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[190]. 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.

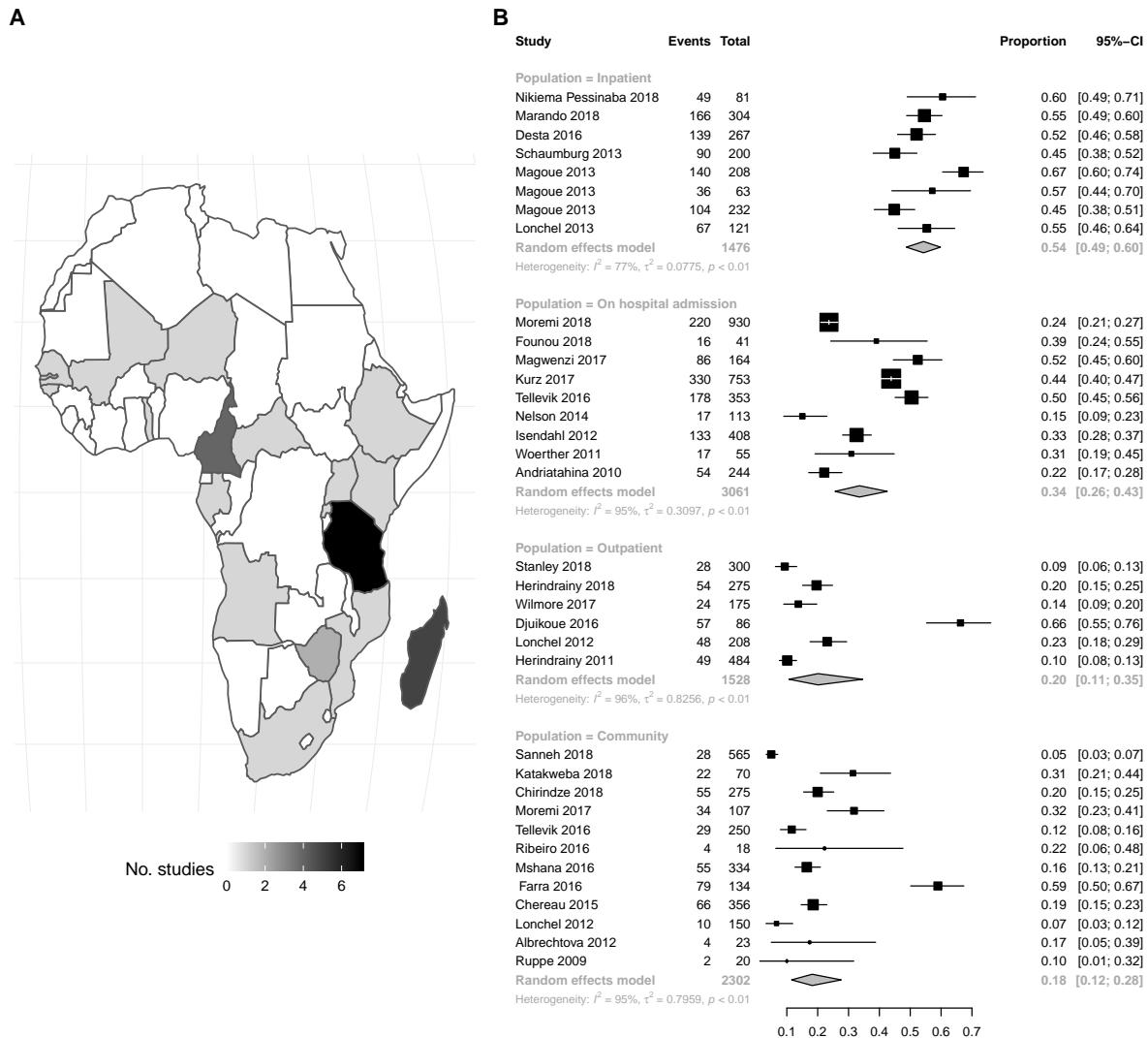


Figure 1.6: ESBL-E gut mucosal carriage in sSA. A: included studies by country; B: forest plot of ESBL-E carriage prevalence stratified by population. Pooled random effect summary estimates shown.

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[194] to 59% in children in the Central African Republic in 2013[169], 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[171,178,194]. 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[193] and Togo[196] 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[196]. Lower socioeconomic status was found to both be protective against ESBL-E colonisation in the Central African Republic[169] and be associated with ESBL-E colonisation in Madagascar[185]; 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[174], and in Harare Zimbabwe, receipt of ART for less than a year was associated with carriage[168]. This relationship is very open to confounding and many studies have not found an association between ESBL-E carriage and HIV infection[171,178,184,192,194,196].

Data on beta lactamase genes 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 [191], the remainder used a variety of PCR techniques[167,179–181,184,186,192].

Only 4 studies are longitudinal cohorts which could provide insight into temporal trends and determinants of carriage[186,188,192,196]; 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

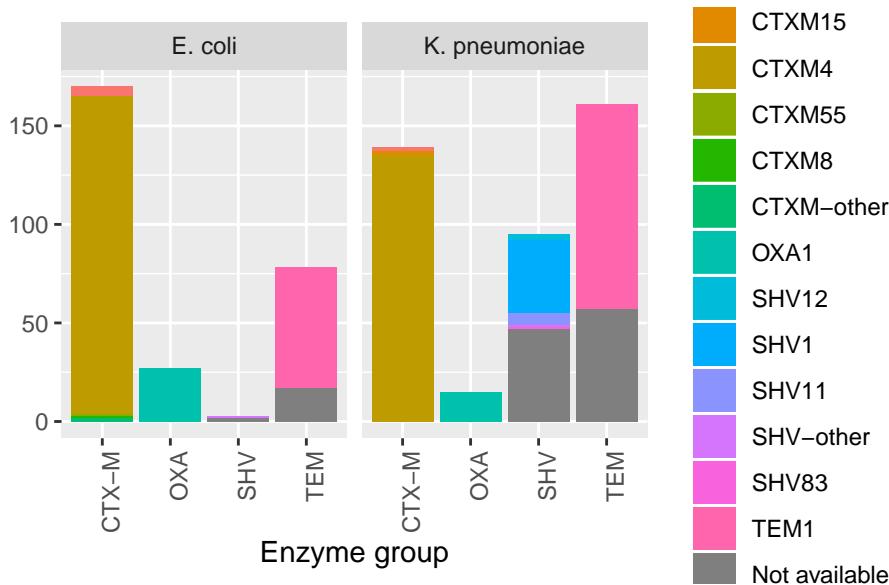


Figure 1.7: Distribution of beta-lactamase genes in carriage ESBL producing *E. coli* (n= 996) and *K. pneumoniae* (n = 607) from 9 studies.

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 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.

It may be that optimising the treatment of severe febrile illness in hospitals is the best place to start to reduce over prescription of broad-spectrum antimicrobials and reduce selection pressure for ESBL-E whilst ensuring timely and appropriate access to the right treatments for those who need them. This is the central hypothesis of this thesis, and the following chapters present the data that can be used to define such a strategy.

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

Chapter 2 (methods) presents the clinical study that forms the basis of the rest of this thesis; given the likely importance of disseminated TB in sepsis in sSA, chapter 3 presents a systematic review and individual patient data meta analysis of prevalence, diagnosis, and mortality hazard of TB bloodstream infection. Chapter 4 presents data on the clinical presentation, aetiology and outcomes of sepsis in Blantyre, Malawi; chapter 5 describes the determinants of long-term carriage of ESBL-E amongst sepsis survivors. Chapter 6 presents an overview of the genomic landscape of ESBL-E in Blantyre, Malawi whilst chapter 7 combines the genomic and epidemiologic data from chapters 5 and 6 to understand mechanisms and drivers of ESBL-E carriage in health and disease in Malawian adults. [microbiome stuff if available]. Finally, chapter 8 provides suggestions of further work.

1.6 Appendix

Table 1.9: Sequential organ failure assessment (SOFA) score

System	Score				
	0	1	2	3	4
Respiratory					
Pao ₂ / FiO ₂ 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 nore- pinephrine < 0.1	Dopamine > 15 or epinephrine > 0.1 or nore- pinephrine > 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	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 lepto-spiro-sis clinic	13 or above referred to central leptospirosis clinic	In house IgM followed by MAT and PCR (acute + conv)	MAT > 1:400 or fourfold rise	51/225 (23%)

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
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%)
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	Phase II IgG IFA titre > 1:128 (acute only)	163/1067 (15%), 10/448 (2.2%) PCR positive

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
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							
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%)

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
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							
Amoako 2018	2016-17	Ghana	2 district 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

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
Sothmann 2017	2012	Ghana	Referral hospital	Febrile Children	PCR	Positive PCR	6/431 (1.4%)
Maina 2016	2011-12	Kenya	District Hospital	Febrile children	IgG ELISA (acute and conv)	Fourfold rise in IgG titre	RF 63/364 (22.4%) 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 health centres	Febrile children	PCR	Positive PCR	42/410 (10.2%)
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	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	Mozam- bique	Not clear	Febrile adults and children	IgG, IgM and PCR (acute only)	Positive PCR	37/99 by PCR (37.4%)
Mugabe 2018	2016	Mozam- bique	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%)

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
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%)
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%)
Chikungunya							
Kayiwa 2018	2014-2017	Uganda	District hospital	Febrile adults and children	PCR	Positive PCR	19/384 (4.9%)

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
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	0/163, IgM 17/163 (10.4%)
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 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

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
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 facilities	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							
Kayiwa 2018	2014- 2017	Uganda	District hospital	Febrile adults and children	PCR	Positive PCR	5/384 (1.3%)

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
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 facilities	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	<i>E. coli</i>	<i>K. pneumoniae</i>
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%)

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	<i>E. coli</i>	<i>K. pneumoniae</i>
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	Buyss	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%)
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%)

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	<i>E. coli</i>	<i>K. pneumoniae</i>
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.

1.7 References

Table 1.2: Sepsis diagnostic criteria

Definition	Diagnosis	Criteria
Sepsis-1 (1991)	SIRS	Two or more of: Temperature $> 38^{\circ}\text{C}$ or $< 36^{\circ}\text{C}$, Heart rate $> 90 / \text{min}$, Respiratory rate $> 20 / \text{min}$ or $\text{PaCO}_2 < 32\text{mmHg}$ (4.3 kPa), White blood cell count $> 12 \times 10^9 / \text{L}$ or $< 4 \times 10^9$ $/ \text{L}$ 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 $> 38^{\circ}\text{C}$ or $< 36^{\circ}\text{C}$, heart rate $> 90 \text{ min}^{-1}$ or $> \text{SD}$ above normal for age, tachypnoea, altered mental status, significant oedema or positive fluid balance ($> 20\text{ml/kg}$ over 24hrs), hyperglycaemia $> 7.7\text{mmol} / \text{L}$ Inflammatory variables: white blood cell count $> 12 \times$ $10^9 / \text{L}$ or $< 4 \times 10^9 / \text{L}$ or $> 10\%$ immature forms, plasma C-reactive protein $> \text{SD}$ above normal, plasma procalcitonin $> 2 \text{ SD}$ above normal Haemodynamic variables: arterial hypotension (SBP < 90 mmHg or MAP $< 70 \text{ mmHg}$ or SBP decrease $> 40\text{mmHg}$ in adults or 2SD below normal range, $\text{SvO}_2 > 70\%$, Cardiac index > 3.5 Sepsis plus organ dysfunction
	Severe sepsis	Organ dysfunction variables: arterial hypoxaemia ($\text{PaO}_2 /$ FiO_2) < 300 , acute oliguria (urine output $< 0.5 \text{ ml kg}^{-1}$ hr^{-1} for at least 2 hours), creatinine increase $> 0.5\text{mg}/$ dL , coagulation abnormalities (INR > 1.5 or aPTT $>$ 60s), ileus, thrombocytopenia (platelet count $< 100,000$ $/\text{mL}$, hyperbilirubinaemia (plasma bilirubin $> 4\text{mg} / \text{dL}$ or $70 \text{ mmol} / \text{L}$
	Septic shock	Sepsis plus hypotension SBP $< 90\text{mmHg}$ or MAP $< 60\text{mmHg}$ or reduction in SBP of 40mmHg from baseline despite adequate volume resuscitation
	Sepsis	Infection plus life threatening organ dysfunction defined by an acute change in SOFA score of 2 or more
Sepsis-3 (2016)	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 1.3: 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 2008-09	Uganda	Severe sepsisc	245 426	95/245 207/426 (39%) (49%)	34 34 (28-41) (27-40)	207/245 362/426 (86%) (85%)	43 63 (11-178) (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.

Table 1.4: 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 1.5: BSI isolates in sepsis in sSA

Organism	N
S. aureus	109
Non-Typhoidal Salmonellae	84
S. pneumoniae	67
Non-salmonellae 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.

Table 1.6: Surviving sepsis campaign guidelines

Recommendation	Strength of recommendation	Quality of evidence
Resusitation		
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

Table 1.7: 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 6hrs 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

Chapter 2

Methods

2.1 Chapter Overview

This chapter gives an overview of the clinical study which underpins this thesis, and the laboratory and computational procedures used in analysis. Further details are given in the individual chapters, where necessary.

2.2 Study site

2.2.1 Malawi

Malawi is a country of 17.5 million people in South-Eastern Africa[248]. It is one of the poorest countries in the world: it is a low income country under the World Bank classification, with a 2017 Gross National Income (GNI) per capita of \$320 in US dollars[249], and was ranked 171st of 189 countries in 2017 by the human development index (HDI), a composite statistic of life expectancy, education and income per capita indicators[(UNDP human development reports)][250]. In 2010, 71% of the population was estimated to survive on less than \$1.90 per day. Life expectancy at birth in 2017 was 63 years, and though significant progress is being made, neonatal and under-5 mortality remains high at 23 and 55 per 1000 live births, respectively. The population is largely rural (83% in 2017), with a young population (44% under the age of 15, 2017) and high fertility rate[249]. Malaria is endemic, and there is an ongoing generalised HIV epidemic: adult HIV prevalence (age 15-49) was estimated to be 9.6% in 2017 (UNAIDS), though falling from a peak of 16.6% in 1999[251]. HIV antiretroviral therapy (ART) national scale up began in 2004 and in 2017 71% of eligible adults and



Figure 2.1: Malawi, showing administrative boundaries (North, Central, and South regions), Lilongwe, the capital city and Blantyre, the study location. Source: openstreetmap.org, used under Creative Commons Attribution ShareAlike 2.0 licence CC-BY-SA

children were estimated to be receiving ART[251]. It is classed by the WHO as a high-TB/high-HIV burden country, with an estimated TB incidence rate of 131 (95% CI 70-210) cases per 100000 population per year[WorldHealthOrganisation2018].

It has a subtropical climate, with three main seasons: a warm wet season from November to April, a cooler dry winter period from May to August and a hot dry period from September to October. Blantyre city, the location of the study in this thesis, is the second city of Malawi with a population of 585000. It is located in Blantyre district, population 995000 in 2018[248], in the Shire highlands at an altitude of 1000m (Figure 2.1).

2.2.2 Queen Elizabeth Central Hospital

Queen Elizabeth Central Hospital (QECH), located in Blantyre city, is the tertiary referral hospital for the Southern Region of Malawi. It has 1300 beds but often operates above

capacity, and is the only site providing free inpatient healthcare to the adult population of Blantyre district. Since 2011 it has had a dedicated emergency department for adults, the Adult Emergency and Trauma Centre, staffed 24 hours a day. Since 2015 (and for the whole of the study period), attendees to the AETC must be referred from a primary health clinic. Adults attending the AETC are triaged by a nurse and then reviewed by a doctor or clinical officer; if admission under a specialty team (including medicine) is deemed appropriate then a patient will be reviewed by an intern or registrar from the admitting specialty and usually by a consultant within 24 hours. There is a 6-bed AETC resuscitation area in which oxygen concentrators, cardiac monitors and a defibrillator are available; none of these items are available in the rest of the AETC.

There are two dedicated single-sex medical wards, each of approximately 60 beds, and one mixed-sex TB ward. Male and female high-dependency units (HDUs), each with a capacity of six beds, have oxygen concentrators (or, if available, oxygen cylinders) to deliver supplemental oxygen. The medical wards are staffed by two or three trained nurses and a variable number of nursing students. Basic nursing care is usually provided by a patients relative or friend, called a ‘guardian.’ Patients on the medical wards are reviewed twice-weekly by a consultant physician and then variably at other times by junior doctors, clinical officers or medical students depending on the availability of staff. Malawi national treatment guidelines suggest ceftriaxone for the treatment of sepsis requiring hospitalisation.

2.2.3 Participating Laboratories

2.2.3.1 Malawi-Liverpool-Wellcome Clinical Research Programme

The Malawi-Liverpool Wellcome Trust Clinical Research Programme was established in 1995 and since then has been active in researching priority health issues in Malawi. It is an affiliate of the Malawi College of Medicine, and is based in the grounds of QECH in Blantyre. It runs an on-site microbiology laboratory which has provided an aerobic blood culture service to QECH since 1998, and also provides CSF microscopy and culture. Bacterial culture is carried out as per British Society of Antimicrobial Chemotherapy (BSAC) guidelines[252] and the laboratory adheres to UK NEQAS external quality control. It is core funded by the Wellcome Trust.

2.2.3.2 Malawi College of Medicine Tuberculosis Laboratory

2.2.3.3 Wellcome Trust Sanger Institute

The Wellcome Sanger Institute is a research institute based in Hinxton, UK, which was established in 1993, and undertakes research in all aspects of genomics including bacterial genomics as part of the parasite and microbes programme. It has one of the largest DNA sequencing facilities in the world as well as high performance computing clusters. It is funded by the Wellcome Trust.

2.3 Clinical Study

The DASSIM (Developing an Antimicrobial Strategy for Sepsis in Malawi) study was an observational cohort study, recruiting from the AETC at QECH with two broad aims: firstly, to describe the presentation, aetiology and determinants of outcome in sepsis in Malawi; and secondly to determineants of carriage of ESBL-E in sepsis survivors.

2.3.1 Entry Criteria

The study was open for recruitment between 19 February 2017 and 2 October 2018; there were three arms. Firstly, in order to define the aetiology of sepsis, adults with sepsis attending AETC were recruited as early as possible in their attendance to QECH following triage. Secondly, in order to clearly define the relative effects of antimicrobial exposure versus hospital admission in ESBL-E acquisition and carriage, antibiotic unexposed adults attending AETC with no plan for antimicrobial administration were recruited. Finally, to define baseline flux of ESBL-E antibiotic-unexposed community members were recruited. Detailed inclusion and exclusion criteria for each arm are given shown in Table 2.1. For logistic reasons, recruitment occurred 7am - 5pm Monday to Friday.

Exclusion criteria were the same for all arms of the study: Participants were not eligible for enrollment if they were unable to give informed consent and no guardian available to provide proxy consent; they spoke neither English nor Chichewa; or they lived > 30km from Blantyre city. The antibiotic-unexposed inpatients and community members were matched on age (+/- 5yr) and sex to sepsis survivors (defined as patients surviving to 28 days). In addition community members were matched on location to sepsis survivors; putative households for recruitment were identified by random walk from the houses of surviving sepsis participants, with initial direction established by spinning a bottle on the floor.

Table 2.1: Study inclusion criteria

Study Arm	Inclusion Criteria
Arm 1 - Sepsis	Adults (16 years or over) attending AETC AND Axillary temperature > 37.5C or history of fever within 72 hours AND Life threatening organ dysfunction defined by any one of: Oxygen saturations < 90 percent on air, respiratory rate > 30 breaths/minute, systolic blood pressure < 90mmHg, glasgow coma scale < 15
Arm 2 - Inpatient	Adults (16 years or over) attending AETC AND No suspicion of infection or plan for antimicrobial administraton AND No antimicrobial therapy within last 4 weeks
Arm 3 - Community	Adults (16 years or over) AND No antimicrobial therapy within last 4 weeks

Note:

AETC = Adult Emergency and Trauma Centre

2.3.2 Study Visits and Patient Sampling

2.3.2.1 Enrollment assessment and first six hours

An overview of the study visit schedule is shown in Figure 2.2. At enrollment, following consent a baseline questionnaire was completed (by the guardian if the participant was obtunded) to capture background demographic and clinical data, a Chichewa-language EQ-5D heath related quality of life questionairre completed, and sample collection undertaken (see below). For hospitalised patients, enrollment aimed to be as soon after triage as possible, and arm 1 (sepsis) patients underwent a more intensive assessment over the first six hours of enrollment. Data on therapies administered over this time period were captured by hourly review by a member of the study team, including the time of administration of antimicrobial therapy

and volumes of intravenous fluid administered, the latter visually confirmed by the study team member. Hourly vital signs and standardised bedside assessment of intravascular status were performed: central capillary refill, limb temperature and passive leg raise. For this latter test, blood pressure and pulse rate were measured with the participant sitting at 45 degrees, and one minute after lying the participant supine and lifting the legs to 45 degrees. All blood pressure measurements were made noninvasively with an automated cuff (Omron M2, Omron, Japan), oxygen saturations measured with a dedicated study pulse oximeter (Contec CM50, Contec Medical Systems, China), and temperature measured in the axilla with a digital thermometer (Omron FWH000, Omron, Japan).

All treatment decisions were at the discretion of the participant's attending healthcare worker.

2.3.2.2 Subsequent visits

Hospitalised participants were briefly reviewed daily Monday to Friday following admission whilst on the wards to capture details of therapies administered. For the 72 hours following admission, vital signs were repeated and an assessment made of intravenous fluid administered; if records of fluid balance were incomplete (as was anticipated to be the case in the majority of cases) then fluid administered was estimated from participant- or guardian-reported number of bags of fluid administered.

The visit schedule for the study is shown in Figure 2.2. Arm 1 (sepsis) and 2 (hospital inpatients) were reviewed by a study team member 7 and 28 days following enrollment, and at 3 and 6 months; community members were reviewed at 28 days and 6 months. At each visit, details of any antimicrobials or other therapies received and any health care facility contact were collected, and the Chichewa language EQ-5D was repeated. Samples were collected as below.

These study visits occurred preferentially at QECH, but at the patient's home if it was difficult for them to attend the hospital. If patients missed a scheduled visit, then attempts were made to contact them by telephone. If these attempts were unsuccessful then attempts to visit them at home were undertaken by a member of the study team.

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

Blood was collected from arm 1 (sepsis) participants only on enrollment and at the 4-week visit. At baseline, blood was collected aseptically directly into collection tubes with a vacutainer device with the following order of draw and volumes: one BacT/Alert (BioMerieux, France) aerobic blood culture bottle (7-10ml), one BD BACTEC Myco/F Lytic mycobacterial culture

bottle (3-5ml) (Beckton Dickinson, United States), one serum (10ml) and two EDTA (4ml, both Grenier Bio-One, Austria) samples. Finger prick for capillary blood was used for point of care diagnostics as described below. At the 4-week visit, 10mls of convalescent serum was collected. At baseline, urine was collected from all arm 1 (sepsis) participants into a sterile polypropylene universal container (Alpha Laboratories, UK) either by the participant themselves (if this was possible) or with the aid of a disposable bedpan.

At the enrollment visit and all other visits for all arms of the study stool was collected into a sterile polypropylene universal container (Alpha Laboratories, UK) with aseptic technique. If it was not possible for a patient to provide stool then a rectal swab was taken using a sterile rayon-tipped swab (Technical Service Consultants Ltd, UK) inserted into 2-3cm into the rectum, rotated for approximately 10 seconds and placed into Amies gel media for transport to the laboratory.

The decision to collect sputum for Xpert testing for tuberculosis or to perform lumbar puncture (LP) rested with the participant's attending healthcare worker. Lumbar puncture, where done, was carried out by QECH AETC or medicine department doctors or clinical officers and 7-10ml of cerebrospinal fluid (CSF) was aseptically collected. Sputum samples were collected in sterile polypropylene universal container and transported to the Malawi College of Medicine (CoM) tuberculosis laboratory. Myco/F lytic bottles for mycobacterial blood culture were also transported to the CoM tuberculosis laboratory; all other samples were transported to the MLW laboratories in the first instance.

2.3.2.4 Imaging: chest x-ray and ultrasound scanning

Ultrasound examination following the FASH (Focussed ASsessment for HIV-associated tuberculosis) protocol[253] was undertaken for all sepsis participants by me, following training with a UK-registered consultant radiologist. The protocol aims to ascertain presence or absence of six features likely to be associated with extrapulmonary TB: pericardial effusion, abdominal lymph nodes, pleural effusion, ascites, hypoechoic focal liver lesions or hypoechoic focal splenic lesions. Scanning was undertaken with a xxxxxxx device with a curvilinear probe; images were saved and 10% of scans were read by a UK-registered consultant radiologist for quality control.

Decision to undertake chest x-ray was at the discretion of the participant's attending healthcare worker. If chest x-ray was done then digital pictures were taken and stored in the study database.

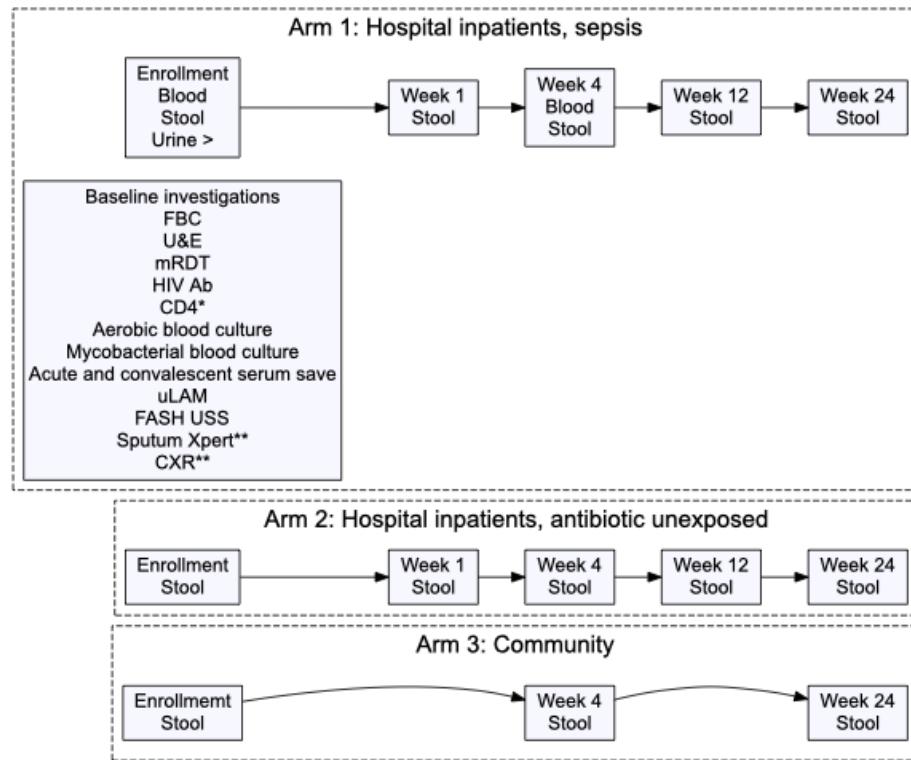


Figure 2.2: Overview of patient sampling schedule. FBC = Full blood count, U&E = Urea and electrolytes, mRDT = Malaria rapid diagnostic test, uLAM = Urinary LAM, FASH USS = Focussed assessment for HIV-associated tuberculosis ultrasound scan

2.3.3 Outcomes and sample size calculations

The two co-primary outcomes for the study are:

- 1) 28 day mortality.
- 2) Aquisition of ESBL-E detected in stool by aerobic culture by 28 days.

Two power calculations were undertaken: to inform the size of the sepsis cohort, and one for the non-sepsis participants. The sepsis component was powered to detect clinically relevant risk factors for death; we made the a priori assumption that a risk ratio of 2 or more is clinically relevant in this setting and patient population. The initial calculation suggested 250 patients with severe sepsis with 50% mortality (as seen in previous studies in Malawi) would have 80% power to detect risk factors for death with prevalence of 20-50% that confer a risk ratio (RR) of 1.5-3; therefore, a RR of 2 is likely to be detected. Logistic considerations resulted in a reduction of a feasible sample size to 225; post-hoc power calculation suggested 80% power to detect risk factors conferring a RR of 2 with 25-50% prevalence, assuming 50% mortality.

For the second co-primary outcome, assuming 30% of participants with sepsis (and hence antimicrobial exposure) acquire ESBL-E by 28 days, and that 50% of them die by day 28, 125 antibiotic unexposed hospitalised participants would give 80% power to detect a 50% relative difference in acquisition. Logistic considerations resulted in the target sample size being reduced to 100 participants; revised post-hoc power calculation suggested that the 80% power to detect a 50% relative difference was maintained.

In order to define the baseline flux of ESBL-E organisms and ESBL genes, one community member for each antibiotic unexposed hospitalised participant, giving a target sample size of 100.

2.4 Diagnostic Laboratory Procedures

Results of all laboratory diagnostic procedures were fed back to participant's attending healthcare worker in real time. If any investigation result became available after the participant was discharged (e.g. Mycobacteraemia was identified) then they were contacted and called back to the hospital to be reviewed by me, and referred for care to the relevant QECH department.

2.4.1 Point of care diagnostics

Point of care tests were carried out on capillary blood for capillary lactate (Lactate Pro 2, Arkay, Japan), for *P. falciparum* HRP-2 antigen (Paracheck Pf, Orchid Biomedical, India) and, if HIV status was unknown, to test for HIV antibodies using Determine HIV 1/2 kit (Abbott Diagnostic Division) and Unigold HIV 1/2 kit (Trinity Biotech Inc.) following Malwian national guidelines[254].

2.4.2 Laboratory diagnostics

2.4.2.1 Haematology and biochemistry

Automated full blood counts (Beckman Coulter HmX Haematology Analyser, Beckman Coulter, USA) were undertaken in the MLW laboratories on EDTA whole-blood samples, as were CD4 cell counts (Becton Dickinson FACSCount, Becton Dickinson, USA) for HIV-infected participants. Serum samples were allowed to settle upright for 30-60 minutes and then centrifuged (at 1300 g for 10 min). Biochemistry testing for urea and electrolytes (Beckman Coulter AU480 Chemistry Analyser, Beckman Coulter, USA) was undertaken immediately and a maximum of three 1.8ml aliquots of serum stored at -80C for later analysis.

2.4.2.2 Aerobic blood and CSF culture

Blood in aerobic culture bottles was incubated in an automated system (BacT/Alert BioMerieux) and identified to species level using the API system (Biomerieux, France) and standard techniques (ref). Anaerobic culture was not available. Coagulase-negative *Staphylococci*, *bacillus spp.*, *diphtheroids* and alpha-haemolytic *Streptococci* other than *S. pneumoniae* were considered as contaminants. Antimicrobial sensitivity testing was undertaken using the disc diffusion method following BSAC guidelines to amoxicillin, chloramphenicol, co-trimoxazole, gentamicin, ciprofloxacin and ceftriaxone.

CSF, where available, was cultured onto blood, chocolate and Sabouraud agar and then identification of any growth undertaken as above.

2.4.2.3 Mycobacterial blood culture

Blood in Myco/F Lytic bottles was cultured at 37C in and inspected daily for the first 14 days with a handheld UK Wood's lamp, and once every two days thereafter. Contents of the bottles were centrifuged (3000xg for 20 mminutes) within 48hr of detection of fluorescence,

examined by ZN and gram stain to exclude contamination, then inoculated into mycobacterial growth indicator tubes (MGIT, Becton Dickinson Diagnostic Systems, United States) for up to 6 weeks. Isolates were classified as *M. tuberculosis* or nontuberculous Mycobacteria by microscopic cording and MPT-64 lateral flow assays (TAUNS Laboratories, Japan).

After 8 weeks of culture of Myco/F Lytic bottles if no fluorescence was seen, then centrifugation and microscopy was carried out. If no organisms were seen, the culture was reported as “no growth.”

2.4.2.4 Sputum Xpert

Sputum Xpert testing (Cepheid, United States) was carried out as per the manufacturers instructions: sputum specimens were mixed with sample reagent and incubated at room temperature for 15 minutes. The liquefied specimen was then loaded into the Xpert MTB/RIF test cartridge for processing.

2.4.2.5 Urinary LAM

Urinary LAM testing was carried out on all available urine samples from HIV-infected participants, using the Alere Deterine TB LAM Ag lateral flow assay (Alere, United States) following the manufacturer’s instructions. Frozen urine samples were used: they were allowed to come to room temperature and then briefly centrifuged to remove debris. 60 microliters of urine was applied to the sample pad and the result read after 25 minutes by comparing to the provided reference scale card. The results were read independently by two readers, with a tie-break read by a third reader in the event of disagreement.

2.4.2.6 Selective stool culture for ESBL-E

Stool and rectal swabs received in the laboratory were stored at 4C pending processing, before being plated onto commercially available ESBL selective media (CHROMagar ESBL media, CHROMagar, France) and cultured aerobically at 37C overnight. Rectal swabs were streaked directly onto the plate, or a cotton tipped applicator used for solid stool. CHROMagar is also chromogenic: *E. Coli* colonies are pink, *Klebsiella spp.*, *Enterobacter spp.* and *Citrobacter spp.* blue and other species white. Blue or white colonies were speciated using the API 20E system. Morphologically distinct colonies were confirmed to be ESBL producers using combination disc methods: the putative ESBL producer was cultured overnight on ISO-sensitest agar with discs of cefotaxime and ceftazidime (30 micrograms) with and without clavulanic acid (10 micrograms), and ESBL production confirmed if there was a difference of at least 5mm

between discs with and without clavulanic acid . For organisms likely to carry a chromosomal AmpC and hence be capable of hydrolysing cefotaxime and ceftazadime, (*Enterobacter spp.*, *Citrobacter freundii*, *Morganella morganii*, *Providencia stuartii*, *Serratia spp.*, *Hafnia alvei*) cefipime (30 micrograms), an AmpC-stable cephalosporin was used with and without clavulanic acid (10 micrograms). Antimicrobial sensitivity testing to meropenam, amikacin, gentamicin, co-trimoxazole, chloramphenicol and ciprofloxacin following BSAC guidelines[252] was undertaken for a subset of confirmed ESBL-producers.

2.4.2.7 Acute and convalescent serologies

PHE stuff

2.5 Molecular methods

One of each morphologically distinct colony from ESBL-E selective culture was taken forward for DNA extraction and whole genome sequencing. DNA was extracted from overnight nutrient broth culture using the Qiagen DNA mini kit (Qiagen, Germany) following the manufacturers instructions. Extracted DNA was shipped to the Wellcome Sanger Institute where it underwent library preparation according to the Illumina protocol and paired-end sequencing on Illumina HiSeqX (*check this*) by the WSI DNA pipelines team.

2.6 Bioinformatics

All analyses were undertaken on the WSI computing cluster running Ubuntu v12.04.2, Precise Pangolin. Quality control of reads for each sample was carried out using metrics provided by the WSI QC pipeline including total number of reads, and statistics obtained by mapping a random 100 Mbases from each sample to a reference and calculating depth of coverage, number of heterogeneous SNPs, GC content and insert size. Kraken[255] v0.10.6, a kmer-based taxonomic assignment tool, was used to assign reads to a species and to identify suspected sample contamination or species misclassification. Reads were *de novo* assembled with SPAdes[256] v3.11.0, with default settings. Assembly metrics of quality (e.g. number of contigs, largest contig, N50 [the contig length on which at least half of the bases in an assembly have been assembled]) were calculated using QUAST[257] v4.6.0 and completeness and contamination of the assemblies assessed by checkM[258] v1.0.7, a tool that uses co-located sets of lineage associated genes to assess genome completeness and contamination.

Contaminated samples (with > 25% contamination as defined by CheckM) or poor assemblies (with N50 < xxxx) were discarded.

Prokka[259] v1.5 with a genus specific database from RefSeq was used to annotate the retained assemblies. The Roary v1.007 pan-genome pipeline[260] was used to construct a core genome with a blastp percentage identity of 95%; genes were considered core if they were present in 99% or more of isolates. A core gene multiple sequence alignment was generated using maaft[261] v7.205, SNP-sites identified using SNP-sites[262] v2.4.1 and the resultant SNP alignment used to build a maximum likelihood phylogenetic tree using IQ-TREE[263] v1.6.3. Trees were visualised in the ggtree v1.14.4 package[264] in R.

Ariba[265] v2.12.1 was used to identify AMR-associated genes using the SRST2[266] database, to identify plasmid replicons using the PlasmidFinder database[267] and to perform *in silico* multi-locus sequence typing (MLST) using the database from <http://mlst.warwick.ac.uk/mlst/dbs/Ecoli> accessed via www.pubmlst.org. *E. coli* phylogrouping was performed with a quadruplex *in silico* PCR using the Clermont scheme[268]. To track carriage of similar bacteria within and between individuals, clustering of the core gene alignment was performed using hierarchical BAPS (bayesian analysis of population structure) algorithm[269], implemented in the rhierbaps package v1.1.0 in R, with 2 levels. To track putative mobile genetic elements (MGE), a difficult task with short-read sequencing, isolates ESBL-gene containing contigs were identified using BLASTn[270] v2.7.0 with and then contigs clustered using cd-hit[271] v4.6 with sequence identity threshold 0.9, word size 5 and accurate mode (command line flag -g) to produce mutually exclusive ESBL-gene contig groupings.

2.7 Statistical Analysis

Details of analysis methods are given in the relevant chapters. All analyses were undertaken using R v3.5.1 and any Bayesian modelling using Markov Chain Monte Carlo (MCMC) methods using Stan v2.18 via the Rstan interface with R. This thesis was written using the bookdown package in R and the code to generate it as well as all analysis scripts are available at <https://github.com/joelewis101/thesis>.

2.8 Study Team

I am the principal investigator of the study, and led a study team which consisted of: Emma Smith, a medical doctor who managed the running of the study from March 2017 - May 2017; study nurses Lucy Keyala, Grace Mwaminawa and Tusekile Phiri who recruited

patients, collected samples and completed follow up visits; field workers Witness Mtambo and Gladys Namacha who recruited patients, collected samples and completed follow up visits; and laboratory technicians Madlitso Mphasa and Rachel Banda who processed laboratory specimens, did stool culture and DNA extractions, and the uLAM testing. The MLW core laboratory staff undertook haematology and biochemistry testing and blood and CSF culture, and the Malawi CoM tuberculosis laboratory staff carried out tuberculosis culture and Xpert testing.

2.9 Data Collection and Storage

Data collection used both the Open Data Kit software (ODK) and structured TeleForm paper forms. Electronic ODK forms were loaded onto Asos ZenTouch tablets running Android using the ODK Collect Android app. Completed forms were pushed daily to the dedicated secure study SQL (structured query language) database built and administered by the MLW data team. Teleform forms were checked by me, batched and scanned by the MLW data team, and variable values automatically extracted by the TeleForm system; validation was undertaken by the MLW data team and data queries generated for missing or invalid values. Queries were resolved either by manually reconciling with the forms or by discussion with the clinical team. Once the data passed validation it was pushed to the SQL database. Completed paper TeleForm records were stored securely in the MLW data department. All data on the study database is stored securely with access restricted to the study PI and the database administrators in the MLW data department. Other paper records including consent forms were stored in a locked cabinet with access restricted to members of the study team. Results of laboratory investigations in the MLW laboratory were stored in the MLW PreLink laboratory information management system (LIMS), anonymised and linked only to the participant unique study ID number. For analysis, anonymised data were extracted from the study SQL and LIMS databases as comma delimited files.

2.10 Ethical Approval, Consent and Participant Remuneration

The study protocol was granted ethical approval by the Malawi College of Medicine Research Ethics Committee (COMREC), protocol number P.11/16/2063 and the Liverpool School of Tropical Medicine (LSTM) Research Ethics Committee, protocol number 16-062. LSTM acted as the study sponsor. All study team member were trained in NIHR Good Clinical Practive (GCP).

All participants in the study provided informed, written consent, as follows: if a patient lacked capacity to provide informed consent themselves then informed consent was sought from the patient's representative, usually their guardian. Patients with no representative and lacking capacity to provide informed consent were not recruited. Initially, eligible patients (or their representative) were approached by a member of the study team and the study was explained to them including study procedures, risks and benefits, financial and confidentiality considerations and how to obtain more information. Written patient information leaflets were provided in English and Chichewa. If the patient/representative was willing to enter the study then they were asked to sign and date two copies of the consent form, and provided with a copy of the form to keep. If either the patient or representative was unable to read then the consent form was read to them by the study team, and witnessed by an additional staff member who is not a member of the study team. If the patient (or their representative) agreed to enter the study, then the witness signed and dated the form. Any patient who was enrolled to the study after consent from a representative and who then regained the capacity for informed consent was then approached independently for informed consent. A patient could withdraw at any time without giving a reason.

Hospitalised patients were not financially compensated for their time and/or transport costs, but all other participants were, following standard MLW guidelines: 500MWK (approximately USD\$0.7 at February 2019 exchange rates) was provided to participants if they were visited at home and 2000MWK (approximately USD\$2.8 at February 2019 exchange rates) or their reported transport costs (whichever was higher) for participants who attended QECH for study purposes.

Chapter 3

Mycobacterium tuberculosis BSI: an IPD meta analysis

Chapter 4

Sepsis in Blantyre, Malawi

4.1 Chapter overview

4.2 Methods

blah blah

4.3 Study population

Figure - Consort diagram

Table - demographics

Table - presentation

Table - health seeking behaviour

4.4 Aetiology

Table

Figure to show crossover

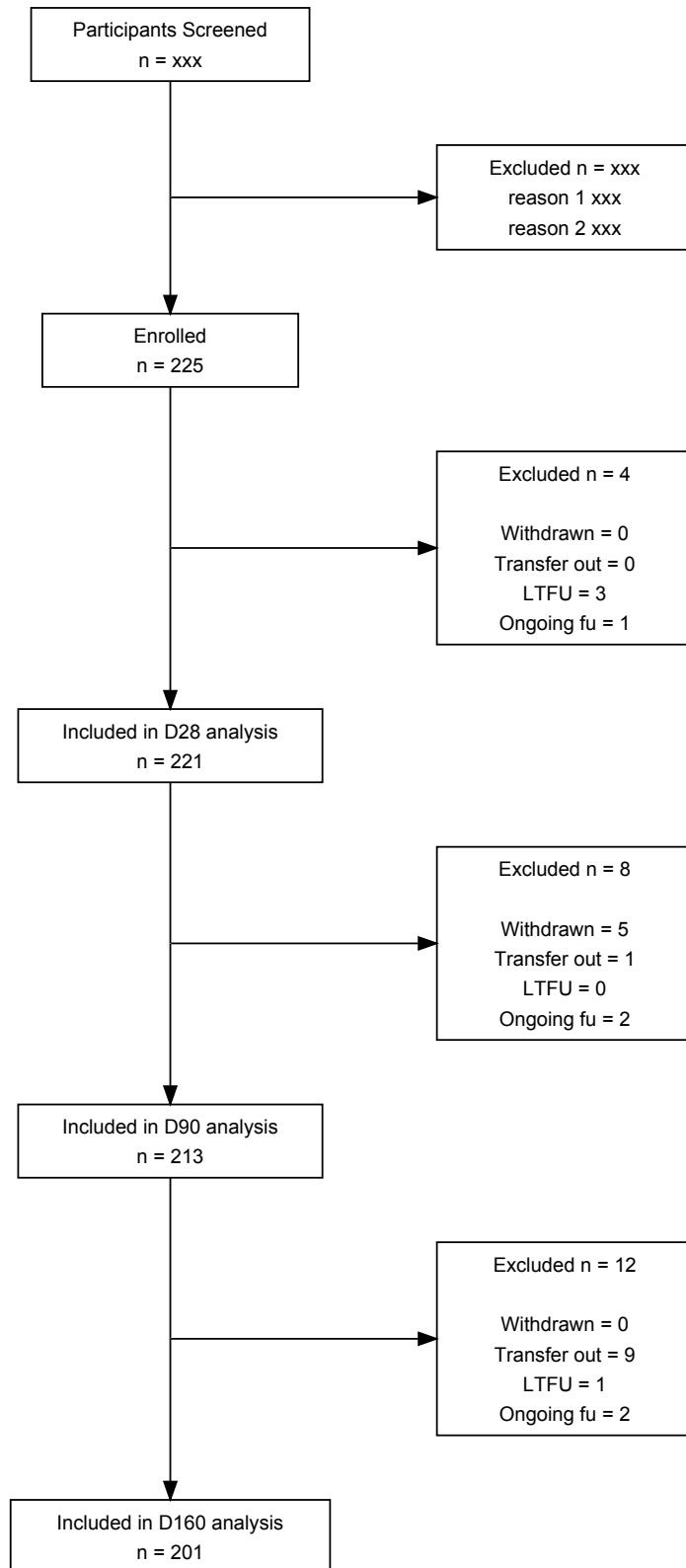


Figure 4.1: Study recruitment and follow up.

4.5 Treatment

Table: Time to antimicrobials Time to fluid Amount of fluid

4.6 Outcome

Table - 28 and 90 day mortality

Figure - KM survival curve

Logistic regression - determinants of 28 day mortality

Chapter 5

Early response to resusitation in sepsis

Chapter 6

Gut mucosal carriage of ESBL-E in Blantyre, Malawi

Chapter 7

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

7.1 Chapter overview

This chapter describes the use of whole-genome sequencing (WGS) of ESBL producing *E. coli* to understand the drivers of gut mucosal ESBL-E carriage. I will begin with a description of the genomic landscape of the isolates from this study: starting with simple descriptions of *E. coli* phylogroup and multilocus sequence type (MLST) I will place the isolates from this study in the context of the *E. coli* population, followed by higher-resolution contextulaistaion using phylogenetics to place isolates from this study in the context of a global *E. coli* collection. I will describe the genetic basis of antimicrobial resistance in these isolates and explore the extent to which AMR genes tend to cluster together beyond what would be expected by chance. Finally, I will attempt to use the resolution offered by WGS to attempt to answer two specific questions: firstly, what is the mechanism of rapid increase in ESBL-E carriage prevalence following hospital admission and antimicrobial exposure we see in this study? Secondly, what is the likely unit of ESBL-E transmission in this study? Are bacteria, or mobile genetic elements (MGE) implicated? And if, MGE, which: plasmids, transposons, integrons - or a combination?

These questions, phrased in this way, seem difficult or impossible to answer given the available WGS data, but by slightly re-framing them they become tractable: first, what is the diversity of apparent hospital-acquired ESBL *E. coli* in comparison to apparent community-acquired isolates? Apparent hospital acquisitions could represent true acquisitions of, for example, a hospital-associated clone - but equally they could be an “unmasking” of minority variant *E. coli* in the microbiota, acquired in the community but not detected by culture because of

low abundance, until enriched for by antimicrobial exposure. If the diversity of apparently hospital acquired isolates is contained within the diversity of community isolates, this would lend support to this latter hypothesis. The second question - what is the unit of transmission in this system - can be re-framed by asking: what is the unit that is most conserved within patients, as compared to between patients? The questions then reduce to a dimensionality reduction problem: in order to address them both, it is necessary to classify either bacteria or MGE into mutually exclusive categories, in order to compare hospital to community isolates, and between-patient to within-patient. I describe the approach I have taken to this below.

7.2 Methods

7.2.1 Bioinformatic pipeline

The basic bioinformatic pipeline used is described in detail in Chapter 2, methods. Briefly, one *E. coli* colony from each patient sample was taken forward for DNA extraction and paired-end short-read whole genome sequencing using Illumina HiSeq X10 at the Wellcome Sanger Institute. Read quality control was undertaken with kraken v0.10.6 and braken v1.0 to assign reads to species[255] and WSI QC pipeline which maps a random 100 Mbases from each sample to a reference and calculates depth of coverage, number of heterogeneous SNPs, GC content and insert size. Samples that contained > 80% non *E. coli*. reads were discarded and *de novo* assembly was undertaken with SPAdes v3.11.0[256]. Assembly statistics were calculated with QUAST v4.6.0[257] and completeness and contamination of the assemblies assessed by checkM v1.0.7[258]. Contaminated assemblies (with checkM-defined contamination of > 25%) or poor assemblies (with less than 1Mb assembled length) were discarded. Annotation was carried out with prokka v1.5[259] with a genus specific database from RefSeq and the roary v1.007 pan-genome pipeline[260] was used to identify a core genome. A core gene multiple sequence alignment was generated using maaft v7.205[261], SNP-sites identified using SNP-sites v2.4.1[262] and the resultant SNP alignment used to build a maximum likelihood phylogenetic tree using IQ-TREE v1.6.3[263], using ascertainment bias correction to correct for the fact that the input pseudosequence contained only variable sites, and using the ModelFinder module used to find the best fitting nucleotide substitution model. This calculates the likelihood of a number of different models and chooses the model with the lowest (best fitting) Bayesian Information Criterion, a statistic which penalises model parameters. Reliability of inferred branch partitions was assessed with 1000 bootstrap replicates. Trees were visualised in the ggtree v1.14.4 package[264] in R.

ARIBA v2.12.1[265] was used to identify AMR-associated genes using the SRST2 database[266],

to identify plasmid replicons using the PlasmidFinder database[267] and to perform *in silico* multi-locus sequence typing (MLST) using the database from <http://mlst.warwick.ac.uk/mlst/dbs/Ecoli> accessed via www.pubmlst.org. The β -lactamase genes *ampC1*, *ampC2* and *ampH* were excluded from the analysis of AMR determinants as they do not usually cause a resistant phenotype in *E. coli*. Because quinolone resistance often results from SNPs in the chromosome in the quinolone resistance determining regions (QRDRs) of the *gyrA*, *gyrB*, *parE* and *parC* genes - rather than acquisition of whole AMR-determining genes, as is the case with the other genes sought by ARIBA - these genes were downloaded from the comprehensive antimicrobial resistance database (CARD, <https://card.mcmaster.ca/>) and ARIBA used to call SNPs in them, with default settings. *E. coli* phylogrouping was performed with a quadruplex *in silico* PCR using the Clermont scheme[268] and *isPcr* v33x2 (<https://github.com/bowhan/kent/tree/master/src/isPcr>)

The rhierbaps v1.1.0 package in R[269] was used to cluster the core genome pseudosequence into sequence clusters (SCs). Two levels were used and these level 2 clusters used to test associations (see statistical analysis, below). To track putative mobile genetic elements ESBL-gene containing contigs were identified using BLASTN v2.7.0[270] of all contigs against the SRST2 database and then contigs containing any given ESBL gene were grouped by the ESBL gene they contained (for example, all *blaCTXM-15* gene-containing clusters were grouped together), and each group clustered using cd-hit v4.6[271] to produce mutually exclusive ESBL-gene-containing contig clusters for each identified ESBL gene. Henceforth, these clusters will be referred to as ESBL-clusters, for brevity. In order to attempt to determine the biological significance of the identified ESBL-clusters (i.e. what kind of MGE element they are likely to represent), basic statistics were plotted (number of samples contained within each cluster, length of longest contig in cluster in kbases, length distribution of all contigs is cluster relative to longest contig and distribution of sequence identity compared to the longest contig in the cluster). Presence of insertion sequences (i.e compound transposons), AMR determinants and plasmid replicons were identified by using BLAST with default settings of each ESBL-cluster representative sequence (as determined by cd-hit i.e one, the longest, for each ESBL-cluster) against the insertion sequence finder (ISfinder) database and the SRST2 database, filtering such that sequence identify was greater or equal to 95%, taking the top hit (as determined by bitscore) for any given location if there were two overlapping hits, and visualising the results in ggenes v0.3.2. To assess lineage association, the ESBL-clusters were mapped back to the core genome phylogeny.

7.2.2 Global *E. coli* collection

In order to place the isolates from this study in a global context, published *E. coli* assemblies were downloaded from the WSI servers. These included 149 ESBL-producing *E. coli* from a single centre study in Chachoengsao province, eastern Thailand[272]. In this study, human clinical isolates from standard care in Bhuddhasothorn hospital were selected on the basis of the ESBL phenotype, and environmental samples were collected as part of a cross sectional study and selectively cultured for ESBL-E in 2014-2015. I also downloaded assemblies of 362 enterotoxigenic *E. coli* (ETEC), selected for an ETEC genomic study from the Gothenburg University ETEC collection to represent a broad collection of ETEC isolated worldwide from 1980-2011[273]; 185 atypical enteropathogenic *E. coli* (aEPEC) sequenced for a study of aEPEC and selected from samples from the Global Enteric Multicentre Study (GEMS) in seven centres in Africa and Asia between 2007-2011[274]; and 94 *E. coli* from QECH in Blantyre, Malawi, a combination of invasive (bloodstream and CSF) and carriage isolates, selected for diversity in AMR phenotype from 1996-2014[275]. Details of the included samples are given in the appendix to this chapter.

Phylogroup and MLST were determined for these context genomes as described above. AMR genes were identified with Ariba and the SRST2 database, as above, and context genomes were classified as ESBL if they contained any Bush-Jacoby group 2be ESBL gene.

7.2.3 Statistical analysis

Ability of presence or absence of resistance determinants to predict phenotypic resistance as determined by antimicrobial sensitivity testing was expressed as sensitivity and specificity, with exact binomial confidence intervals. In order to explore clustering of AMR genes, the Jaccard index was calculated for a given AMR-gene pair using the jaccard v0.1.0 package in R. The Jaccard index, a measure of the similarity of two sets of data, is defined as *intersection over union*; in this context, for a given pair of AMR genes x and y , the Jaccard index $J(x, y)$ is the number of isolates that contain both gene x and y divided by the total number that contain either x or y . By definition it lies between 0 (x and y never co-occur) and 1 (x and y always co-occur). Co-occurrence matrices using the Jaccard index were plotted using the pheatmap v1.0.12 package in R. The statistical significance of co-occurrence of genes was assessed by generating 2x2 contingency tables for a given gene pair and p values generated using a Fisher's test with Bonferroni correction; a p value of less than 0.05 was considered statistically significant. Co-occurrence networks of genes occurring commonly together (defined as Jaccard index > 0.5) at a rate greater than expected by chance ($p < 0.05$ following Bonferroni correction) were plotted using igraph v1.2.2 and ggraph v1.0.2 in R.

To explore hospital or community associations of any given *E. coli* clade, the location of isolation was first plotted against the phylogenetic tree; location of isolation was classified as hospital, community, or recent hospital discharge (defined as a date of isolation within 2 weeks of hospital discharge). This latter category was used because it is possible that a patient could acquire an ESBL-E clone in hospital but only be sampled once leaving hospital; using only hospital isolated and community isolated categories could therefore introduce bias. Hospital or community association of each sequence cluster was assessed using a Fisher's exact test of proportion of hospital associated samples (defined as sum of hospital isolated and recent hospital discharge) for the given sequence cluster as compared to proportion of hospital associated samples in the remainder of the samples, with a Bonferroni correction for multiple comparisons. $p < 0.05$ was again considered statistically significant.

To compare within-patient to between-patient conservation of bacteria (as represented by core genome alignment and sequence cluster) and ESBL-containing MGE (as represented by the ESBL-clusters) several approaches were taken. Firstly, I assessed whether either sequence cluster or ESBL-cluster were conserved within an individual at all. I hypothesised that any within-patient correlation is likely to be a function of time: samples closer together in time may be more likely to be similar. To assess if this was the case for bacteria, pairwise core genome pseudosequence SNP distance was calculated using snp-dists v0.4 (<https://github.com/tseemann/snp-dists>) for all samples and plotted against the time difference (in days) between samples, within and between patients, and with a smoothed curve fitted using a general additive model with cubic splines. Because of significant overplotting, this was also plotted as a 2D density plot. Based on these plots, the within and between patient SNP distances were compared in two post-hoc defined groups binned by time distance between the samples (50 days or less vs. more than 50 days), and distributions compared with Kruskal-Wallace tests.

I then compared the within patient temporal clustering of ESBL-clusters and sequence clusters, by estimating the proportion of within-patient samples that contain the same ESBL-cluster or sequence cluster, as a function of time; essentially a temporal auto-correlation function. To estimate this, I considered pairwise comparison of all within-patient samples. For any given time between samples, t I defined a window of $+/-5$ days and estimated the probabilities as the number of all within-patient sample pairs in the window $[t - 5, t + 5]$ that contained the same sequence cluster or ESBL-cluster divided by the total number of all within-patient sample pairs within that time window. Exact binomial confidence intervals for these proportions were generated and probabilities plotted as a function of time. In order to estimate the probability of two samples containing the same sequence cluster or contig-cluster purely by chance, 1000 sample pairs were randomly drawn from all samples with replacement and the proportion of these samples that contained the same sequence cluster or ESBL-cluster calculated.

Finally, to inform the question as to what the likely unit of transmission in this system is, I assessed what was most conserved within patients, in pairwise sample comparison: bacteria (as represented by core gene sequence cluster), ESBL-containing MGЕ (as represented by ESBL-cluster), or both. Simple proportions in all-against-all pairwise comparison - stratified by whether between-patient or within-patient - were calculated: the proportion of samples that contain the same core gene sequence cluster only, the proportion of samples contain the same ESBL-cluster only, and the proportion that contain both sequence cluster and ESBL-cluster. Proportions were compared between within and between-patient strata in these three groups using Fisher's exact test, with $p < 0.05$ considered statistically significant.

7.3 Results

7.3.1 Samples and quality control

In total, 519 *E. coli* underwent DNA extraction and were shipped from Malawi to WSI; these represented all sequential isolates at the time of final DNA extraction, which occurred in two batches in February 2018 and October 2018. Kracken/Bracken read assignment of these samples is shown in Figure 7.1. The majority of samples have $> 90\%$ of reads assigned to *E. coli*; a minority have $< 90\%$ of reads assigned to *E. coli* but a very closely related species such as *Shigella*, and as such are likely to be pure *E. coli* culture with read misclassification. However, 12 samples have $> 80\%$ reads assigned to a non- *E. coli* species such as *Klebsiella pneumoniae*. These samples were assumed to represent upstream species misidentification or, perhaps more likely, selection of the wrong sample from the freezer archive for culture and DNA extraction, given that for any sample ID there are often several bacterial species identified and cryopreserved. These samples were excluded from further analysis.

Of the remaining 507 samples, there were a median (IQR) of 2339594 (2112842.5-2533930.5) reads, with a median (IQR) depth of coverage (obtained by mapping a random 100Mbases to a reference *E. coli* genome, Escherichia coli strain K-12 substrain MG1655, NCBI reference NC_000913.3) of 58 (51-66). One sample had an order of magnitude lower number of reads (291556) with depth of coverage 0; this was assumed to represent sequencing failure and it was excluded from further analysis.

The output from quast and checkM are shown in Figure 7.2, where N50 (the minimum contig length upon which at least half assembled bases are contained) is plotted as a function of total assembled length. The expected *E. coli* genome length is around 4.6Mb and most samples cluster close to this at a total assembled length of $\sim 5\text{Mb}$. However it is clear that some assemblies have failed, with low N50 and low assembled length. It is also apparent that some

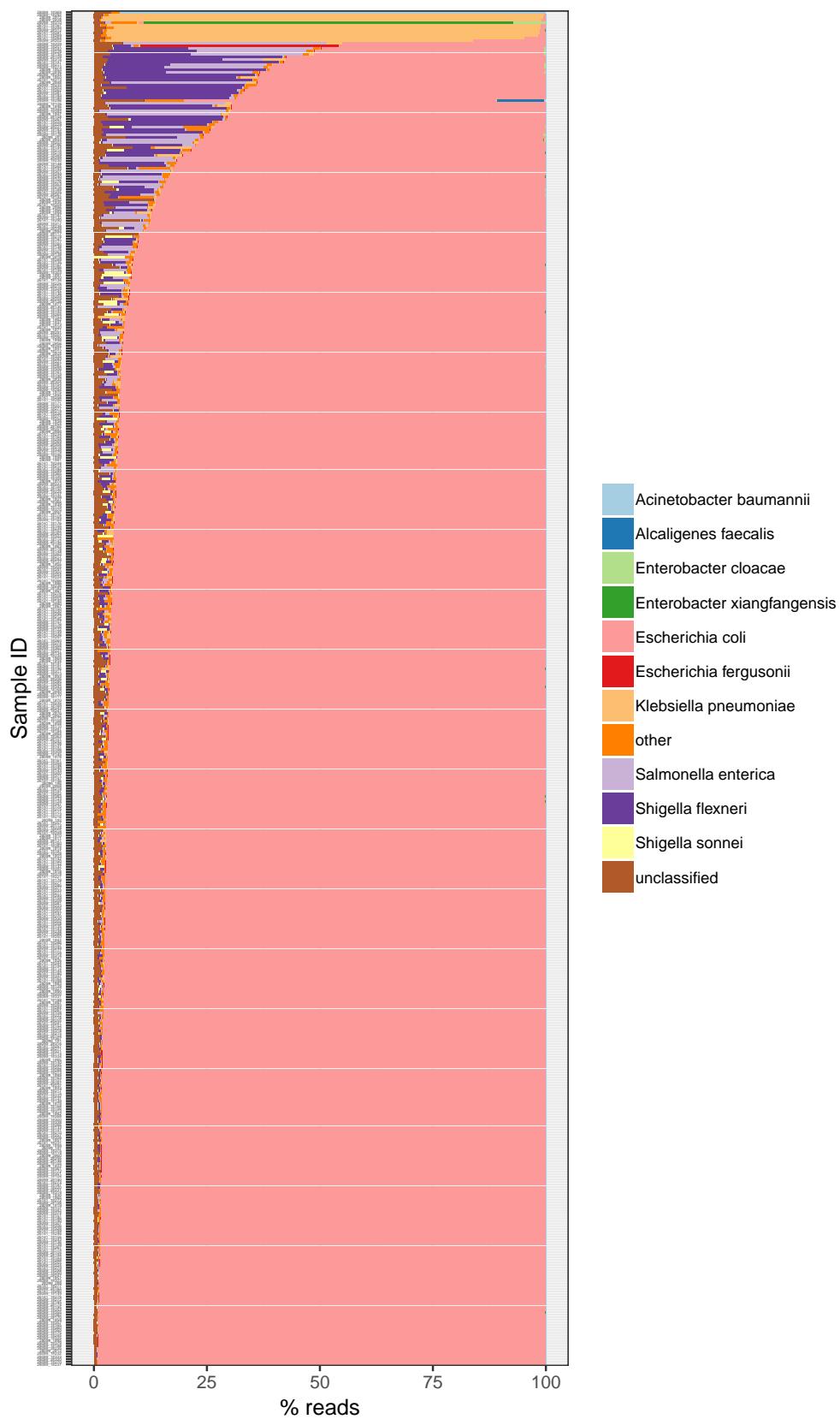


Figure 7.1: Species read assignment of all samples

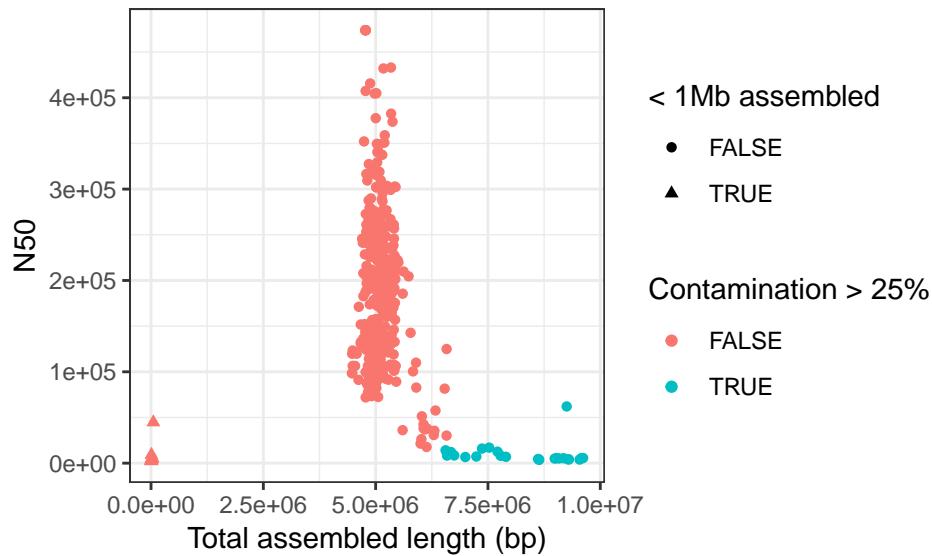


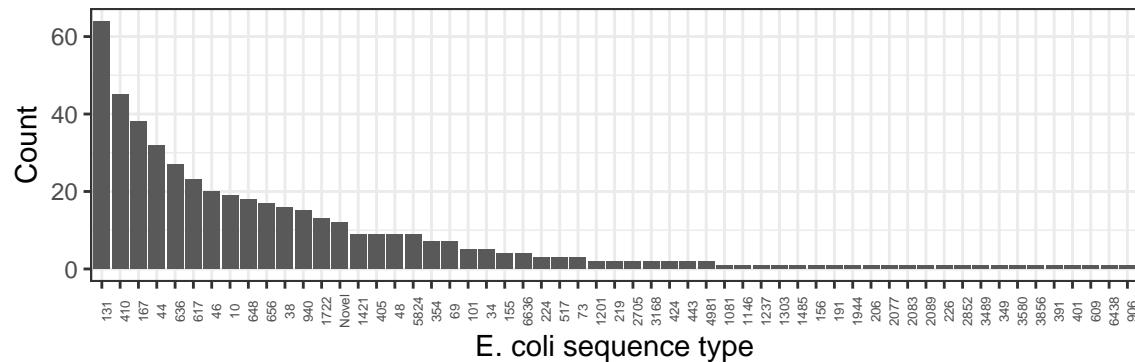
Figure 7.2: N50 as a function of total assembled length. Failed assemblies with less than 1Mb assembled shown as triangles. Contaminated assemblies with checkM-defined contamination above 25% shown in blue.

samples seem to be contaminated, as indicated by low N50 and much longer than expected total assembled length. Defining assembly failure as < 1Mb assembled length (triangles in the plot, n = 9) and contamination as checkM-defined contamination of > 25% (blue points in the plot, n = 24) and excluding both groups results in 33 further samples being excluded from further analysis.

In total, therefore, 46/519 (9%) of samples which were submitted for sequencing were excluded from downstream analysis. The remaining 473 samples represent 69% (474/686) of the cultured *E. coli* in this study, and were recovered from 230 participants. 354 are from patients with sepsis, 86 are from hospitalised inpatients and 33 are from community members, with a median of 2 (range 1-5) samples per participant. N50, total assembled length and number of assembled contigs are shown in the appendix to this chapter.

7.3.2 Phylogroup, MLST and core genome phylogeny of study isolates

The commonest *E. coli* phylogroup was phylogroup A: 204/473 (43%) samples belonged to phylogroup A, followed by phylogroup B2 (96/473 [20%]), F (53/473 [11%]), B1 (43/473 [9%]) and C (43/473 [9%]) and D (26/473 [5%]). Two samples were Clade I or II (so called cryptic clades) and 6/473 (1%) were unknown phylogroup using the Clermont PCR scheme. In the MLST analysis, 56 recognised sequence types (STs) were identified, and 12 samples were novel STs; however over half (249/473 [53%]) of samples were represented by the top seven most

Figure 7.3: *E. coli* multilocus sequence type distribution

frequent STs (Figure 7.3). ST131 was the most commonly isolated sequence type (64/473 [14%] of isolates) followed by ST410 (45/473 [10%] of isolates) and ST167 (38/473 [8%] of isolates).

The Roary pan-genome pipeline identified a core genome in the study isolates of 2966 genes, with a pan-genome of 26840 genes. The resultant core gene pseudosequence of length 1388742 bases contained 99693 variable sites, which were used to infer the maximum likelihood phylogenetic tree. The IQTREE ModelFinder module determined that a general time reversible (GTR) model with FreeRate site heterogeneity with 5 parameters provided the best fit to the data. The inferred tree is shown in Figure 7.3 along with isolate phylogroup and sequence types; in general, as expected, sequence types were largely monophyletic and phylogroups tended to cluster together.

7.3.3 Study isolates in a global context

The global collection of *E. coli* comprised 1273 samples, including the 473 from this study. 753/1253 (60%) were from Africa, 335/1253 (27%) from Asia and 167 (13%) from South America. The majority of samples, 1026/1253 (82%), were from stool, with 106/1253 (8%) truly invasive samples from blood or CSF and 63/1253 (5%) possibly invasive samples from urine, pus, or sputum. 65/1253 (5%) of samples were environmental, all from Thailand. 670/1253 (53%) of samples contained at least one ESBL-encoding gene. The majority of isolates with ESBL gene (622/670 [92%]) came from this study or the Thai ESBL study. Phylogroup A was the commonest phylogroup in the global collection (482/1273 [38%]), followed by B1 (333/1273 [26%]) and B2 (191/1273 [15%]); phylogroup C was uncommon in the global collection (74/1273 [6%]) but the majority of the phylogroup C samples came from this study (43/74 [58%]). All of these 43 phylogroup C isolates belonged to a single ST, ST410; this ST was not seen at all in the previous Malawian study of largely invasive

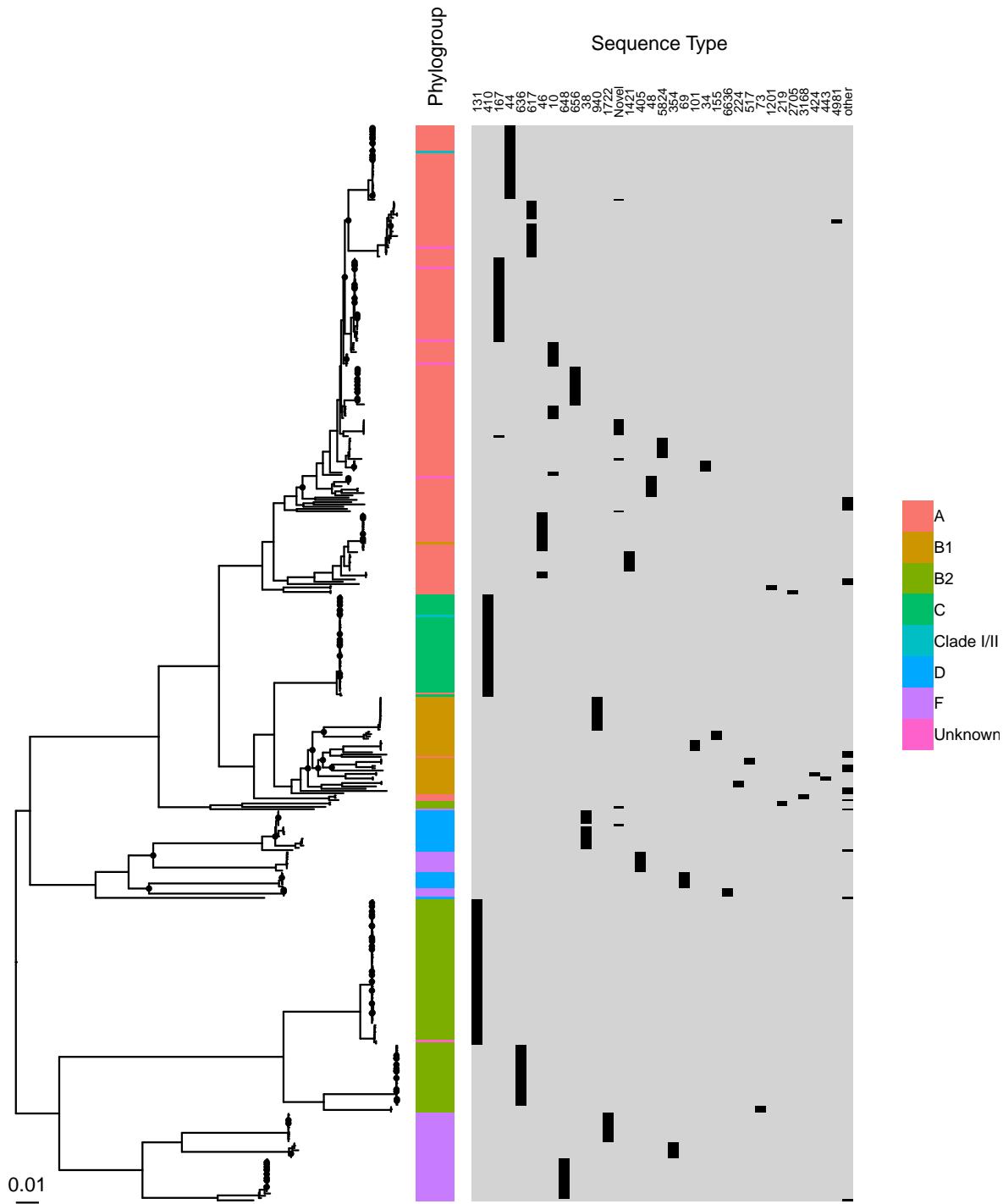


Figure 7.4: Maximum likelihood phylogenetic tree of included study *E. coli* isolates showing phylogroups and sequence types. Bootstrap support of less than 90% is indicated by a black circle at a given node. Scale bar indicates 0.01 SNPs/site.

isolates, despite being the second-commonest ST in this study, and was unusual in the global collection (11/800 [1%] ST410 in global collection vs 43/473 [9%] in this study). Similarly, the third-commonest ST in this study, ST167, was not seen at all in the global collection. However, ST131, the commonest ST in this study, was again the commonest ST in the global collection.

The Roary pan-genome pipeline identified 2872 core genes in a pan genome of 44840 genes; this large pan-genome is consistent with the open *E. coli* pan genome that will continue to increase in size as isolates are added. The core gene alignment contained 604817 bases with 77194 variable sites, which were used to infer the maximum likelihood phylogenetic tree, using same nucleotide substitution model as previously.

The inferred tree is shown in Figure 7.5). Isolates from this study are distributed throughout the tree, and there is widespread mixing of isolates from diverse geographic regions. Though invasive isolates are spread throughout the tree, there is a tendency for them to cluster together, particularly in phylogroup B2, a phylogroup with has a recognised association with ExPEC[276]. The Malawian ST410 and ST167 isolates clustered tightly together, but by comparison, ST131 isolates from this study were distributed among ST131 isolates from other studies, both in Malawi and elsewhere (Figure 7.6).

7.3.4 Antimicrobial resistance determinants

All identified AMR genes are shown in Figure 7.7A, alongside a summary of number of isolates with resistance mutations to given antimicrobial classes (Figure 7.7B) and the phenotypic resistance of the isolates for which phenotypic antimicrobial resistance testing was carried out (449/473 [95%]). The isolates contained a median (IQR) of 16 (12-17) resistance genes, and 100 different resistance alleles were identified in total. A description of resistance gene by class, along with a consideration of concordance (or otherwise) of phenotypic resistance and predicted resistance from genotype, are given in turn below.

7.3.4.1 β -lactam resistance

All isolates contained at least one gene that conferred resistance to third-generation cephalosporins, either an ESBL gene (n= 472) or a carbapenemase (n=1). The majority of ESBL-gene containing isolates contained only one ESBL gene (459/472 [97%]); fewer contained 2 (13/472 [3%]) and none contained more than 2. *bla_{CTX-M}* was the commonest ESBL gene, and over two thirds (319/473 [67%]) of isolates contained *bla_{CTXM-15}*. ESBL *bla_{SHV}* (26/473 [5%] of isolates) genes were also seen. ESBL *bla_{TEM}* (1/473 isolates) and

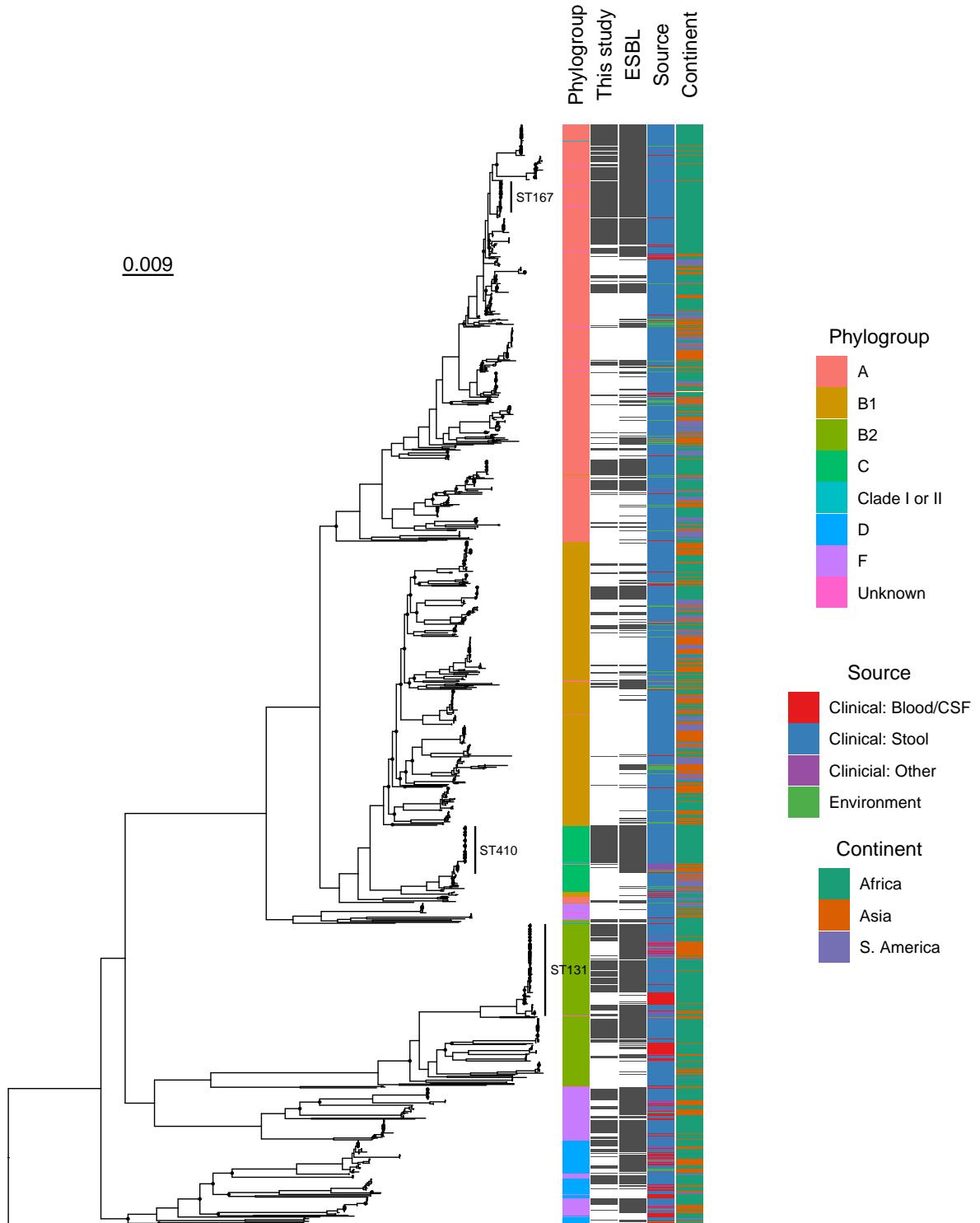


Figure 7.5: Midpoint rooted maximum likelihood phylogenetic tree of included study *E. coli* isolates along with global context isolates, showing phylogroups, source sample type and continent of isolation (coloured bars). Dark grey bars indicate isolates from this study or isolates with ESBL gene presence, as labelled (this study or ESBL, respectively). Three most frequently isolated STs in the current study (131, 410 and 167) labelled. Bootstrap support of less than 90% is indicated by a black circle at a given node. Scale bar indicates 0.009 SNPs/site.

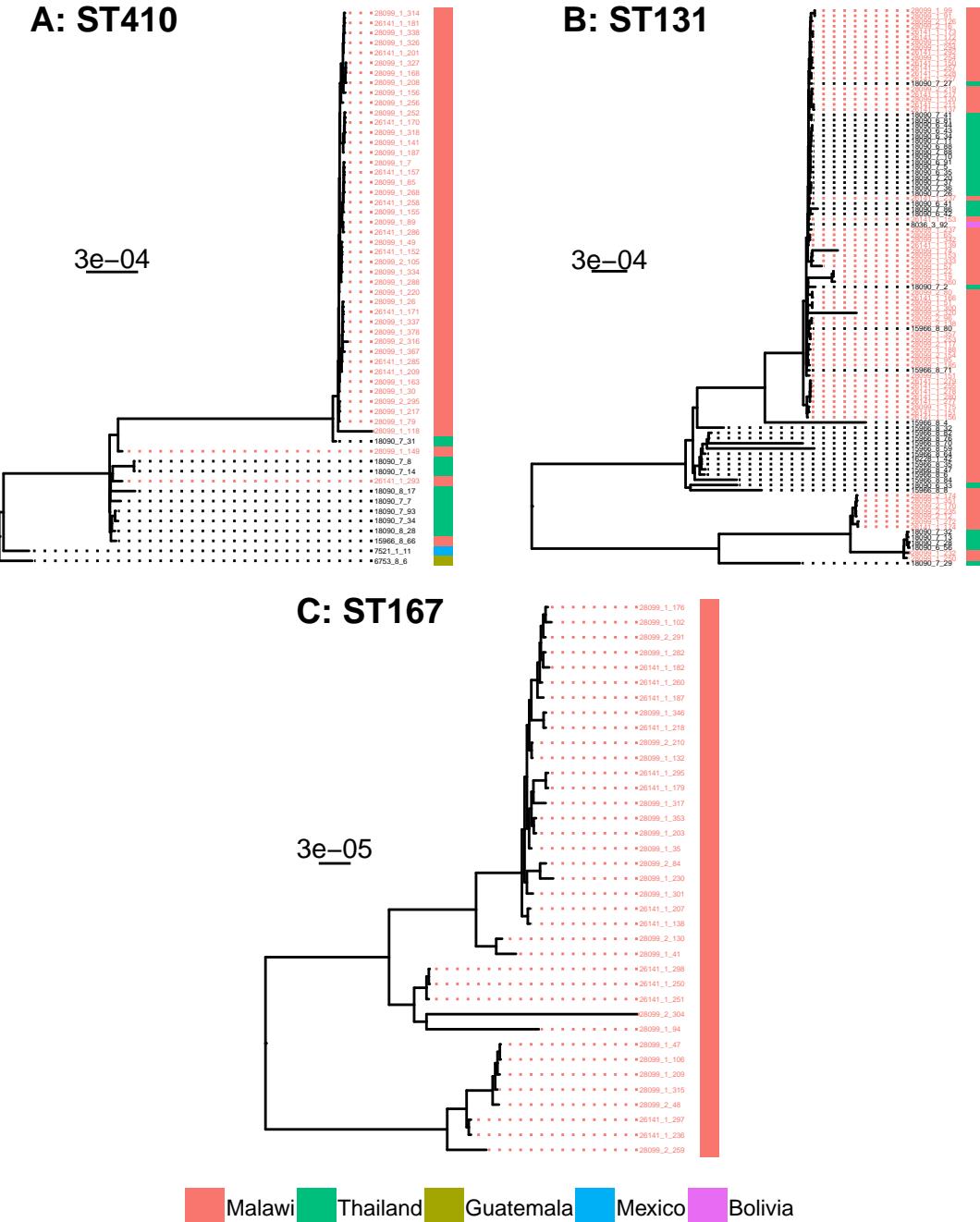


Figure 7.6: Subtree of ST410 (A, left) and ST131 (B, right), and ST167 (C, bottom) showing multiple introductions of ST131 into Malawi, in comparison to a single introduction clonal ST410 and ST167 clades. Colour of tree tip label indicates isolation from this study (red) or other studies (black), and coloured heatmap indicates country of isolation. Note that the scale bar in C is an order of magnitude different from A and B.

bla_{OXA} (1/473 isolates) were very unusual; however, narrow spectrum *bla_{TEM}* and *bla_{OXA}* β -lactamases were common: *bla_{OXA-1}* and *bla_{TEM-95}* were present in 186/473 [39%] and 289/473 [61%] of isolates respectively. Plasmid-mediated *bla_{ampC}* genes were identified in 45/473 (9%) of isolates, almost all (44/45) *bla_{CMY-42}*; this was unexpected as all of these isolates were confirmed to be ESBL-producers by combination disc testing. This testing uses cephalosporin-containing discs both with and without clavulanic acid, and confirms EBSL production by a difference in zone size between these discs, as ESBL enzymes are inactivated by clavulanic acid. However, the cephalosporins used in this test are likely to be hydrolysed by *ampC* enzymes, and if these isolates were producing such enzymes it could confer cephalosporin resistance regardless of the presence or absence of clavulanic acid. This was not the case for any of these isolates; none of them hydrolysed the cephalosporins used in the presence of clavulanic acid. It may be that the *bla_{CMY}* genes were not expressed.

The carbapenemase gene identified was a *bla_{NDM-5}*; the isolate harbouring this gene was recovered from the stool of a 67-year old man with no history of foreign travel nor hospitalisation. He had been admitted to the hospital with fever seven days previously and treated with seven days of intravenous ceftriaxone for sepsis, the source of which was not clear. He made an uneventful recovery, and no carbapenemase-containing isolate was recovered from his stool at any other time. The *bla_{NDM-5}* gene was carried on a partially assembled IncX3 plasmid. BLAST of this assembly against the NCBI database showed that this contig had 99% sequence identity with a previously sequenced pNDM-MGR194 46.2 kbp *bla_{NDM-5}* containing Inc-X3 plasmid found in India between 2011-13[277]. We fully assembled the plasmid by mapping reads back to pNDM-MGR194 with Burrows-Wheeler alignment and found it to be extremely similar, with only 13 SNPs compared to pNDM-MGR194.

7.3.4.2 Quinolone resistance

108/473 (23%) of isolates contained plasmid-mediated quinolone resistance PMQR genes, either *qnr* or *qep*. Nonsynonymous mutations were identified in at least one of the quinolone resistance-determining regions (QRDR) - *gyrA*, *gyrB*, *parC*, or *parE* - in 349/449 (78%) of isolates. The majority of mutations were well-described QRDR mutations (codon 83 and 87 in *gyrA*, codon 80 and 84 in *parC* and codon 458 in *parE*, Figure 7.8A). QRDR mutations tended to cluster together (Figure 7.8B) but alone they correlated poorly with phenotypic resistance. Of the 449 samples with available phenotypic sensitivity data, 294/449 (65%) were intermediate or resistant to ciprofloxacin, but 349/449 (78%) had a mutation in any codon in one of the four QRDR; presence of any QRDR mutation together with presence of PMQR had sensitivity of 95% (95% CI 93-98%) but specificity of 27% (95% CI 20-34%) for phenotypic quinolone resistance. Presence of mutations at all of codon 83 and 87 of

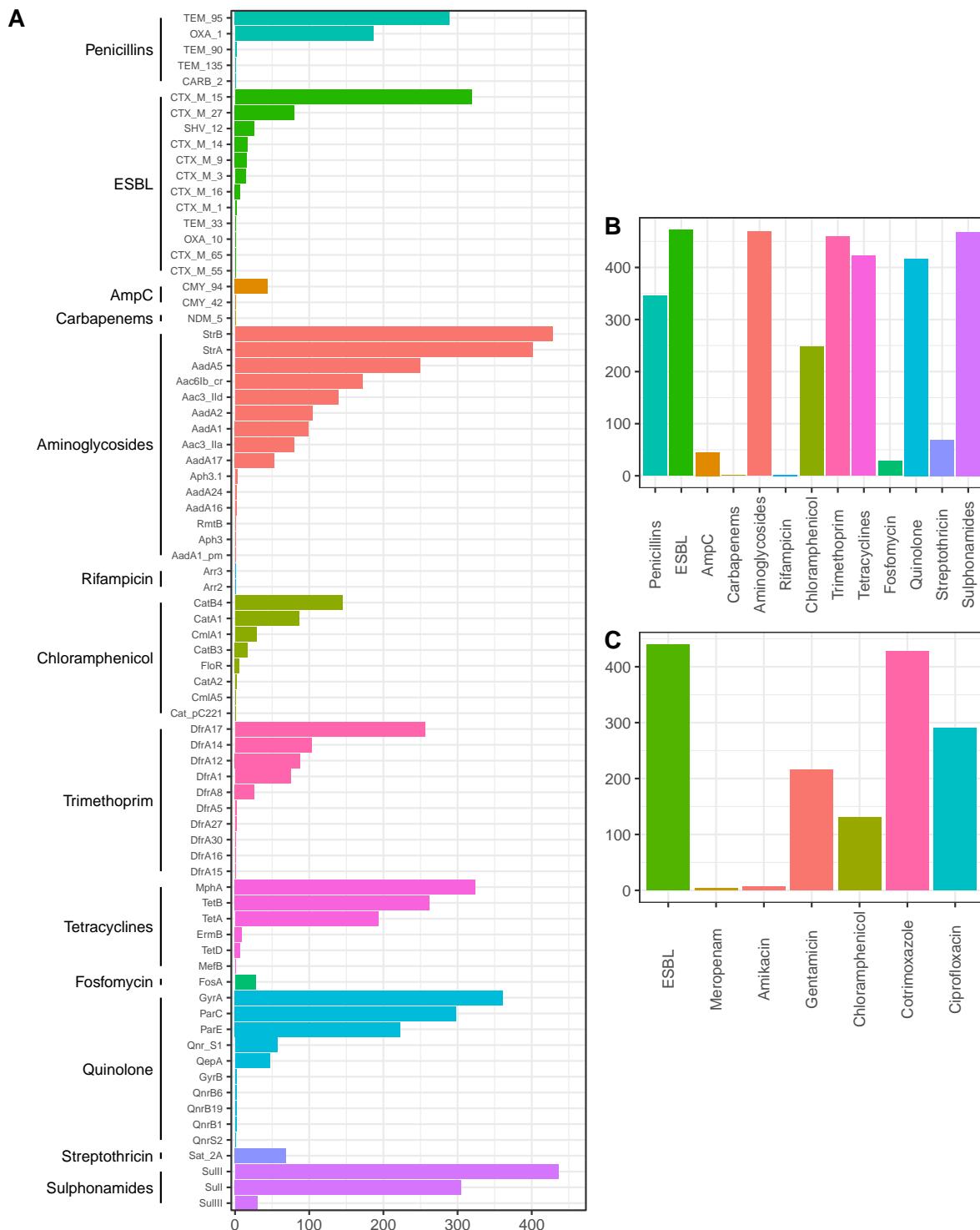


Figure 7.7: A: Frequency distribution of AMR genes identified in isolates. Class of antimicrobial to which gene confers resistance is shown. B: Number of isolates with any mutation to a given class. Any mutation that could possibly confer resistance to a given class is included, including any mutation in the QRDR for quinolones. C: Phenotypic resistance patterns for subset of samples in this analysis that also underwent phenotypic testing (n = 449)

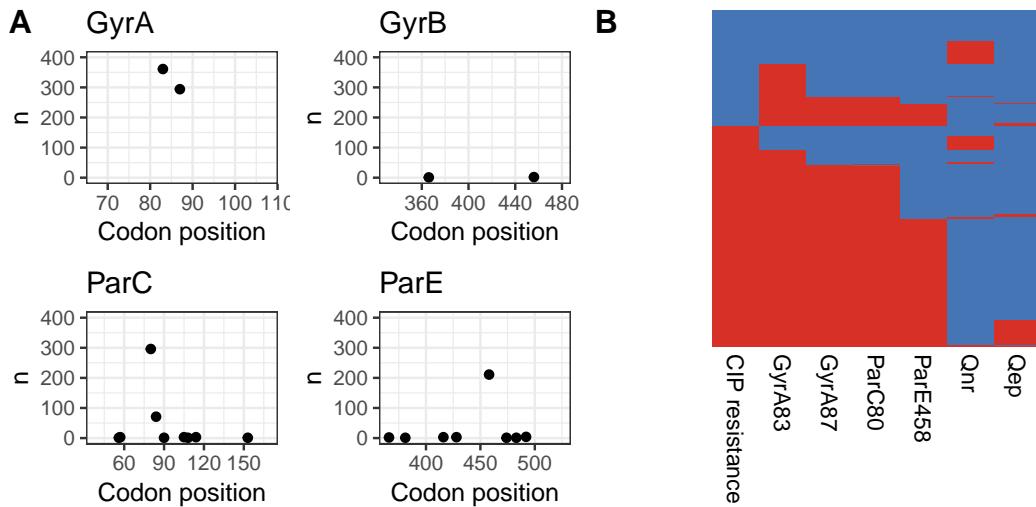


Figure 7.8: A: Mutation positions in quinolone resistance-determining regions, showing that most mutations are well-recognised (see text for details) B: Co-occurrence heatmap of QRDR mutations (*gyrA*, *parC*, or *parE*) plasmid-mediated quinolone resistance mutations (*qnr* or *qep*) and phenotypic resistance. Each row is one sample, red = presence, blue = absence.

gyrA and at codon 80 of *parC* has previously been shown to have the best predictive ability of phenotypic resistance[278], and this showed improved, but still poor, discrimination for phenotypic resistance with sensitivity 89% (95% 85-93%) and specificity 54% (95% CI 46-62%) in this dataset.

7.3.4.3 Aminoglycoside resistance

Aminoglycoside resistance genes were very common in the sequenced isolates, with 469/473 (99%) of isolates containing at least one aminoglycoside gene, and most containing multiple different genes: median number of aminoglycoside resistance genes per isolate was 4 (IQR 3-5). Despite streptomycin being absent from all Malawian treatment guidelines save for re treatment of tuberculosis, the streptomycin resistance genes *strA*, *strB* and *aadA* family of genes (also called *aad(3")*) were very commonly seen (Figure 7.9A). Genes that would be expected to confer gentamicin resistance - *aac(3)-IIa*, *aac(3)-IId* and *aac(6')-Ib-cr* were common, but genes that would be expected to confer amikacin resistance (*rmtB*) and kanamycin resistance (*aph(3')*) were unusual (Figure 7.9B)[279,280]

The predictive value of presence of *aac(3)-IIa*, *aac(3)-IId* or *aac(6')-Ib-cr* for phenotypic gentamicin resistance was moderate at best with sensitivity 77% (95% CI 71-83%) and specificity 73% (95% CI 67-79%). Of 6 phenotypically amikacin resistant or intermediate isolates, all had recognised streptomycin resistance determinants (*strA*, *strB* or *aadA*) but

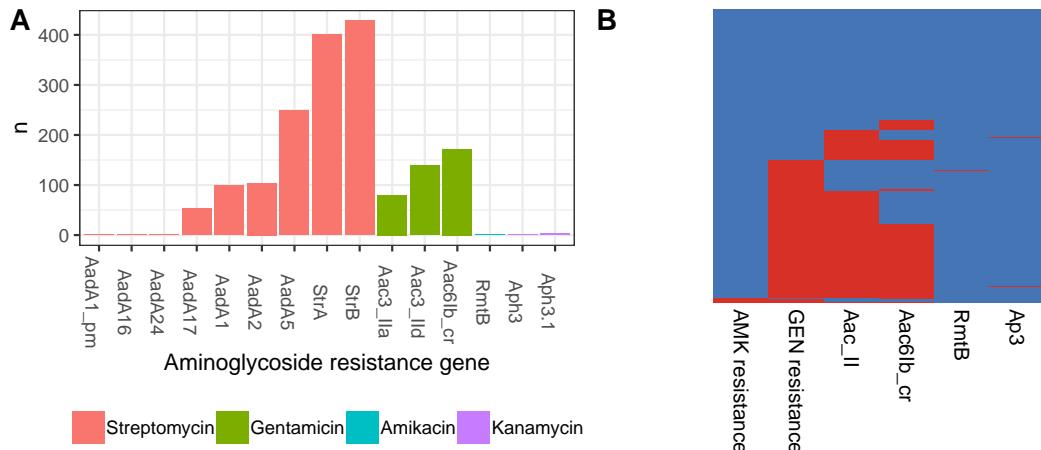


Figure 7.9: A: Aminoglycoside mutations and expected resistance to gentamicin, amikacin and kanamycin B: Heatmap showing phenotypic amikacin and gentamicin resistance and identified resistance genes that could be expected to confer resistance to these agents (see text for details). *Aac_II* in heatmap indicates presence of either *aac(3)-IIa* or *aac(3)-IIa*. Each row is one sample, red = presence, blue = absence.

4/6 had no other aminoglycoside determinant identified. Of the remaining two, one isolate contained *aac(6')-Ib-cr* and one both *aac(3)-IIa*, *aac(3)-IId*.

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

248/473 (52%) of isolates contained at least one chloramphenicol resistance gene (Figure 7.7), usually 1 (210/248 [85%]), less commonly 2 (37/248 [15%]) or 3 (1/248 [<1%]). *catB4* was the most commonly identified gene but once again phenotypic chloramphenicol resistance correlated poorly with presence of chloramphenicol resistance genes (7.10A)) with presence of any chloramphenicol resistance gene predicting phenotypic resistance with a sensitivity of 70% (95% 62-78%) and specificity of 55% (95% CI 49-60%).

Almost all isolates contained either a trimethoprim resistance (459/473 [97%]) or a sulphonamide resistance gene (468/473 [99%]); only 3/473 isolates did not contain either. Trimethoprim resistance genes were all of the *dfrA* family; *sulII* was the commonest sulphonamide resistance determinant (Figures 7.7 and 7.10B). Summary sensitivity of presence of any *dfrA* or *sul* gene as a predictor of phenotypic resistance was 100% [95% CI 99-100%] but (partially due to the rarity of co-trimoxazole sensitivity in this dataset) specificity was 13% [95% CI 2-40%].

Tetracycline resistance genes were also very common, identified in 422/473 (89%) of isolates,

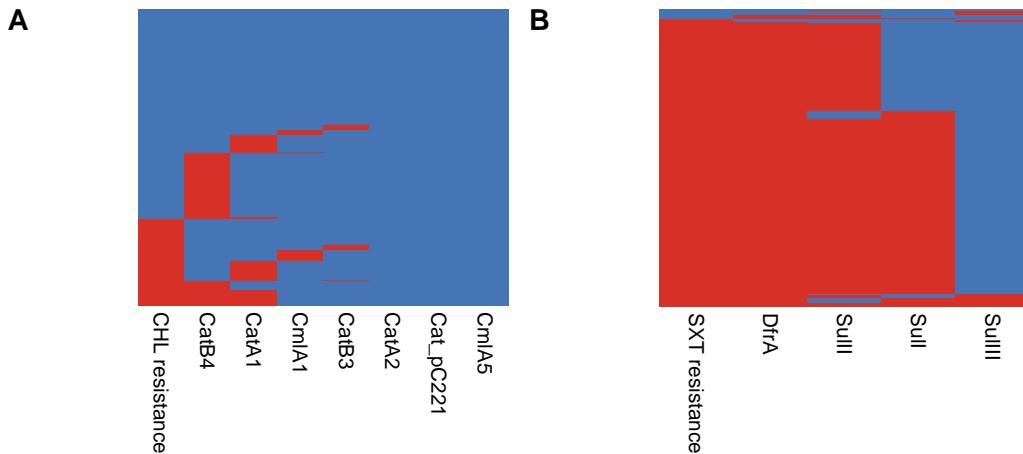


Figure 7.10: Heatmap showing phenotypic chloramphenicol (A) and cotrimoxazole (B) resistance and identified resistance genes that could be expected to confer resistance to these agents. Each row is one sample, red = presence, blue = absence

most commonly *mphA* (324/473 [68%] of isolates), followed by *tetB* (262/473 [55%] of isolates) and *tetA* (193/473 [41%] of isolates). No antimicrobial sensitivity testing was carried out for any agent of the tetracycline class. Resistance determinants for rifampicin (*arr2* and *arr3*) were rarely identified, in 2 isolates and the *sat2* gene, conferring resistance to streptothrinicin (a nucleoside antibiotic with no clinical compounds in use) was seen in 69/473 [15%] of isolates; the significance of this is unknown. Finally, the fosfomycin resistance determinant *fosA* was seen in 28/473 [6%] of isolates, despite this antimicrobial being unavailable in Malawi.

7.3.4.5 Clustering and lineage association of AMR determinants

Next, I explored associations of AMR determinants, both with each other in an attempt to identify putative clusters that could represent mobile genetic elements (MGE) that could be tracked within and between patients, and with lineages of the phylogeny. There was clear clustering of AMR genes beyond what would be expected by chance (Figures 7.11A and B), including clustering of the ESBL gene *bla_{CTXM-15}* with penicillinases *bla_{OXA-1}* and *bla_{TEM-95}*. Though some identified clusters correspond to known MGE (e.g. the *sulII-strA-strB* cluster[281]), there was a clear lineage association of certain gene combinations on mapping the presence or absence of AMR determinants back to the phylogeny (Figure ??C), meaning that these AMR-gene associations likely represent a combination of co-location on MGE and confounding by association with lineage.

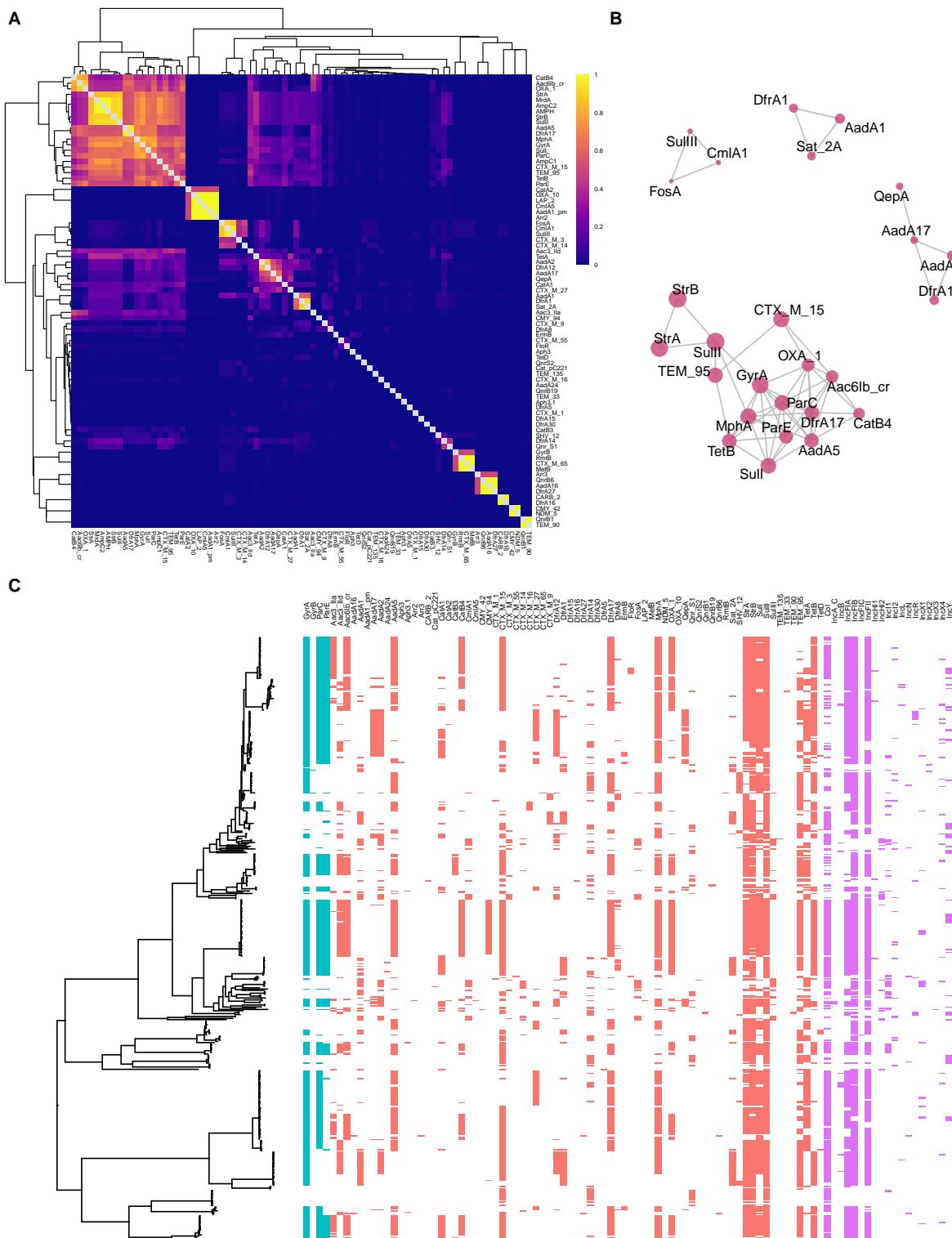


Figure 7.11: A: Row and column clustered heatmap of pairwise Jaccard index matrix, showing clustering of AMR genes. B: Networks of commonly (jaccard index > 0.5) and significantly ($p < 0.05$, Bonferroni corrected) co-occurring AMR genes. C: AMR genes mapped back to midpoint rooted maximum likelihood phylogenetic tree, showing lineage associations of genes.

7.3.5 Plasmid replicons

Presence or absence of the identified plasmid replicons is shown mapped to the phylogeny in Figure 7.11C. IncFIb was most commonly identified (399/473 [84%] of isolates), followed by IncFII (383/473 [81%] of isolates) and IncF1a (324/373 [68%] of isolates). Col plasmids were also frequently identified, in 308/473 [65%] of isolates. Once again, there seems to be some lineage associations of presence or absence of replicons.

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

Finally, in order to test metadata associations of bacterial lineages or MGE, I used several techniques: considering core gene SNP distance between isolates to infer continuous carriage and/or transmission events, and clustering core gene pseudosequences and ESBL-containing contigs into mutually exclusive groups which can then be used to test associations. Below, I first describe the outcomes of the clustering algorithms used, before describing tests of association with metadata.

7.3.6.1 Hierarchical BAPS clustering of core gene pseudosequences

The hierarchical BAPS algorithm clustered the core gene alignments into 15 level one (top level) clusters, denoted sequence clusters A-O, and a total of 48 level two (lower level) clusters, denoted sequence clusters 1-48 that were almost exclusively monophyletic and often corresponded closely to the multilocus sequence types (STs, Figure 7.12A). Intracluster pairwise SNP distance varied (Figure 7.12B) but the clusters were often reasonably clonal: SC6, SC8 and SC23, for example (the three largest clusters) had median (IQR) intragroup pairwise SNP distance of 62 (34-97), 326 (18-378) and 18 (11-24) respectively.

7.3.6.2 ESBL-clusters

The 473 samples contained 486 ESBL genes (Figure 7.13A); 5 genes only occurred once in the collection and so no attempt was made to cluster them. Of the remaining 481 genes pairs, BLAST failed to identify the ESBL-gene containing contig in 2 samples (one in which ARIBA had identified *bla* – *CTXM* – 15 one *bla*_{CTXM-27}), but identified the remaining 479 ESBL genes on 478 contigs, with perfect agreement with ARIBA as to which AMR gene was present in which sample. Only one contig carried two ESBL genes: *bla*_{CTXM-3} and *bla*_{CTXM-15}; the remaining 477 contigs contained one. The *cd-hit* algorithm grouped the 477 unique contigs

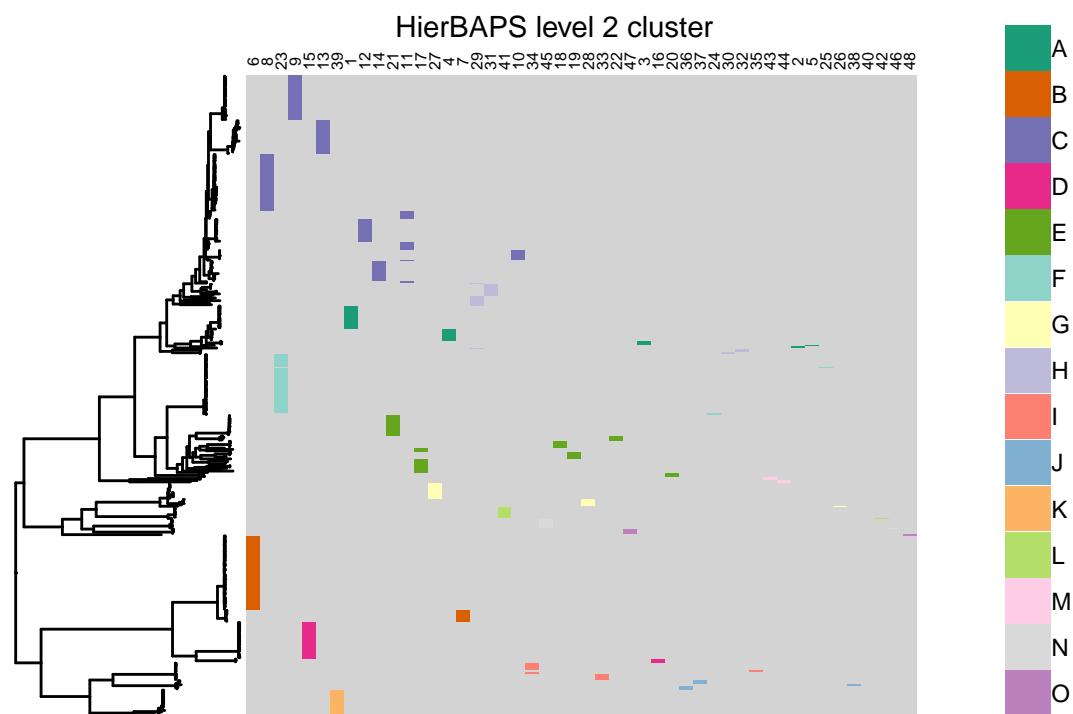
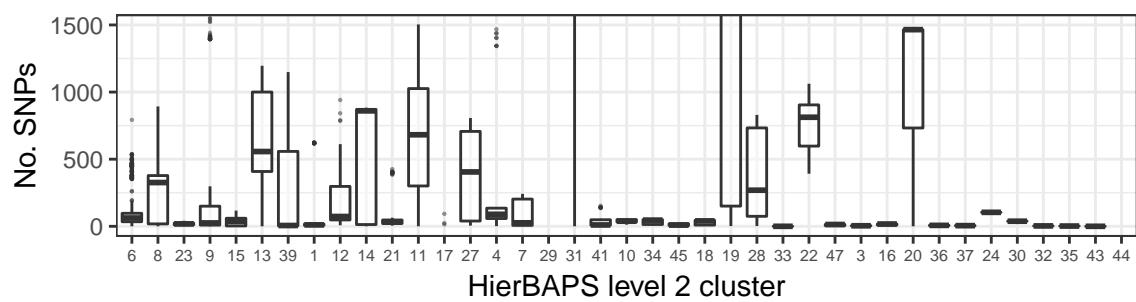
A**B**

Figure 7.12: A: Core gene hierarchical BAPS clusters mapped back to phylogeny. Heatmap shows level 2 (lower level) with colour denoting level 1 (top level) cluster membership. B: Intracluster pairwise SNP distance for level 2 sequence clusters. Axis restricted to 0-1500 SNPs and as result SC17 (median 6881 SNPs), SC29 (median 2970 SNPs), SC31 (median 2970 SNPs) and SC44 (median 12322 SNPs) boxes are not shown. Boxplots show median and IQR, whiskers show 1.5 times IQR, and outliers are points falling beyond whiskers.

into 99 clusters (Figure 7.13B). In total, over 90% of the ESBL-genes (432/479 [90%]) were contained in the 52 largest contig clusters.

The *cd-hit* algorithm selects one member of a cluster (the longest) as the representative. The structure of these representative contigs was explored in an attempt to understand type of MGE they were likely to represent. The length of the representative clusters was very variable, ranging from 1.8kbp to 905.8kbp, with median (IQR) 46.1kbp (11.1-215.5kbp). The other cluster members were usually fragments of these representative contigs with varying sizes - a median (IQR) 60% (36-100%) of the representative contig length - but had high sequence identity, median (IQR) 100.0% (99.7-100.0%) (Figure 7.17 in the appendix to this chapter).

I then explored the insertion sequence (IS), AMR gene and plasmid replicon content of the representative contig for each cluster using BLAST against the SRST2, ISfinder and Plasmidfinder databases (Figures 7.18 and 7.19 in the appendix to this chapter). Every ESBL gene was closely associated with at least one IS, commonly ISEcp1, IS26 and IS903B. IS26 was frequently associated with an apparent 108bp fragment of a *catB4* chloramphenicol resistance determinant. Some ESBL-genes were associated with particular IS; *blaCTXM-15*, *blaCTXM-9* and *blaCTXM-1*, for example were very commonly associated with ISEcp1, whereas *blaSHV-12* was associated with IS26. ESBL genes were not infrequently associated with other resistance determinants, including commonly *blaCTXM-15* with *blaTEM-95*. Plasmid replicons were occasionally identified, including an IncFIB plasmid carrying *blaCTXM-15* and an IncQ1 plasmid carrying *blaCTXM-27*. It is clear that the same configuration of AMR genes and IS are seen across different contigs, despite a varying backbone, implying historical transposition events. Finally, to assess lineage associations of the identified ESBL-clusters, I mapped the clusters back to the tree, and found that there was a strong lineage association (Figure 7.13C).

7.3.6.3 Assessing for healthcare-associated lineages

Having clustered bacteria and MGE using *hierBAPS* and *cd-hit* respectively, I then mapped the location of sample collection back to the phylogeny and used the *hierBAPS* SCs to assess for healthcare associated lineages (Figure 7.14). In general, healthcare-associated isolates were distributed throughout the tree and across all SCs, rather than there being a clear hospital-associated lineage. The exception to this was SC23, corresponding to MLST 410, which was slightly more likely to be healthcare associated. When comparing the proportion of healthcare associated samples within each SC to the remained of samples, SC23 had a statistically significantly increased proportion of healthcare associated samples on Fisher's exact test ($p = 6.3 \times 10^{-4}$, threshold of significance following Bonferroni correction 1.0×10^{-3}), though it was by no means health-facility restricted: 50% (21/42) of SC23 samples were isolated in the community.

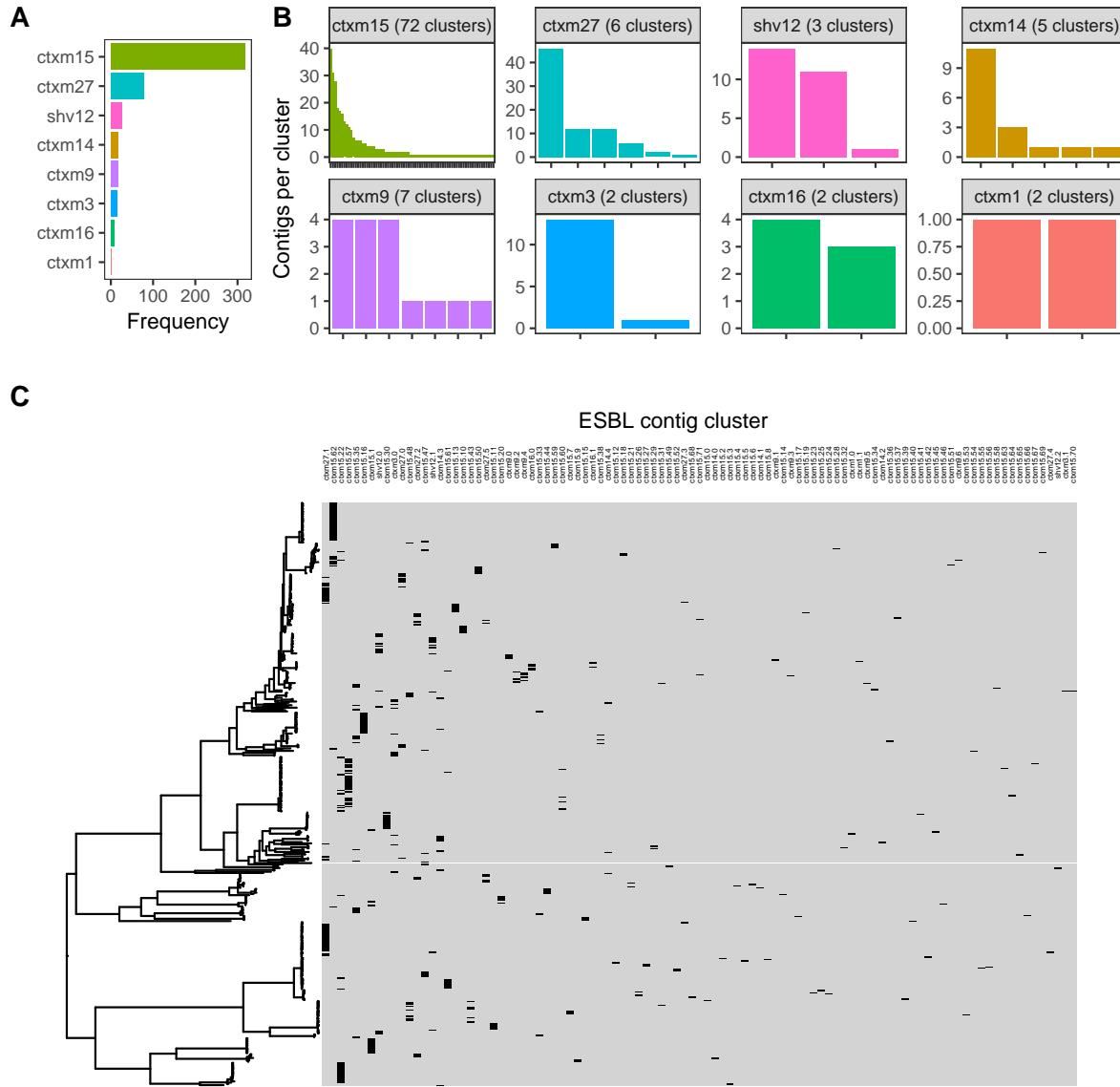


Figure 7.13: A: Frequency distribution of ESBL genes in included samples. B: Frequency distribution of samples per ESBL-cluster, stratified by gene. C: ESBL-cluster membership mapped back to phylogeny.

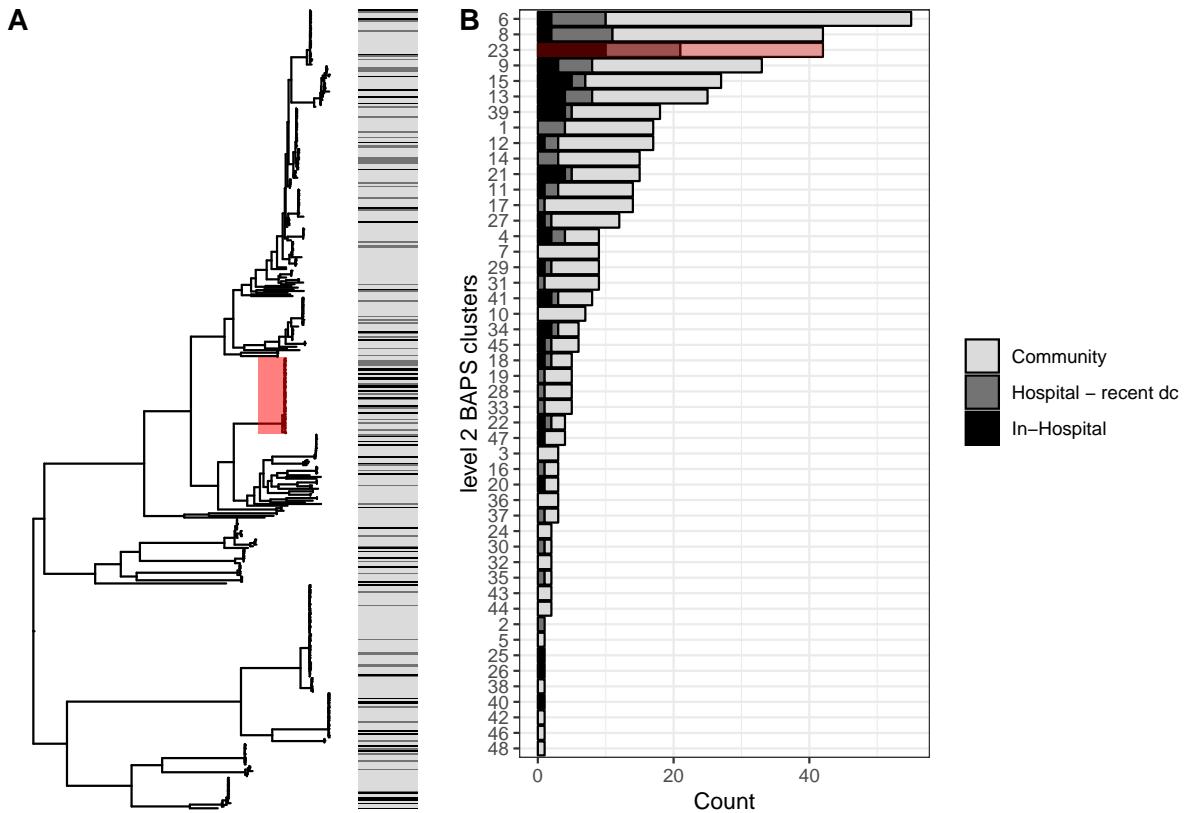


Figure 7.14: A: Location of sample isolation mapped back to phylogeny B: Distribution of location of sample isolation stratified by hierBAPS cluster. In each case, community isolates include those cultured from samples collected on the first day of hospital admission, in-hospital isolates are from patients who have been hospitalised > 24 hrs and recent discharge isolates are from patients who have been discharged from hospital within the last 2 weeks. Sequence cluster 23, highlighted in red, showed a statistically significant association with hospitalisation (see text).

7.3.6.4 Assessing for within-patient conservation of lineage or MGE

To answer the question as to what elements (bacteria or MGE) are conserved within individuals across time I first compared all-against-all pairwise SNP distance between and within patients; first as a scatter plot, and then, because of significant overplotting, as a density plot (Figure 7.15). This suggested that there are a cluster of points close to the origin in the within-patient plot that are not seen in the between-patient plot: before approximately 50 days, there are more similar within-patient isolates than seen in the between-patient isolates. Dichotomising time at 50 days (based on inspection of the density plots) and performing a Kruskal-Wallace test found a statistically significant difference between the before 50 day and after 50 day pairwise SNP distance distribution in the within patient stratum ($p = 0.008$) but not in the between-patient stratum ($p = 0.07$). After 50 days, the distribution of between- and within-patient SNP distances are similar ($p = 0.45$). However it is clear from the plots that even at small t and within-participant, there is significant diversity in the SNP distances, and that some isolates close together in time, within the same participant, are only distantly related.

Having confirmed that there is a signal for within-participant temporal conservation of ESBL-E, I then sought to determine if the sequence clusters and ESBL-clusters were similarly conserved over time, and if so, which was the more conserved. The proportion of pairwise within-patient samples that contained the same ESBL-cluster and sequence cluster were significantly greater than would be expected by chance when the time between the samples is less than 35 days for sequence cluster and 32 days for ESBL-cluster (Figure 7.16A). After this time, the lower confidence interval of the sequence cluster and ESBL-cluster curve crossed the proportion of samples that would be expected to be the same by chance, suggesting that, after 35 or 32 days, the chance of any two within-patient samples having the same sequence cluster or ESBL-cluster (respectively) is the same as if the two samples were randomly picked from the data set without regard to patient. The two curves have a very similar appearance; to address the question of which element is most conserved within an individual - sequence cluster, ESBL-cluster, or both - I performed an all-against-all pairwise comparison of which elements were conserved (Figure 7.16C), and found that only ESBL-cluster and sequence cluster together are conserved within patients at a significantly greater proportion than between patients ($p = 1.1 \times 10^{-12}$).

7.4 Discussion

In this chapter, I have presented the results of whole genome sequencing of 473 ESBL *E. coli* recovered from serial sampling of 230 Malawian adults from a combination of healthcare-associated and community settings, in an attempt to understand drivers of ESBL-E carriage. I

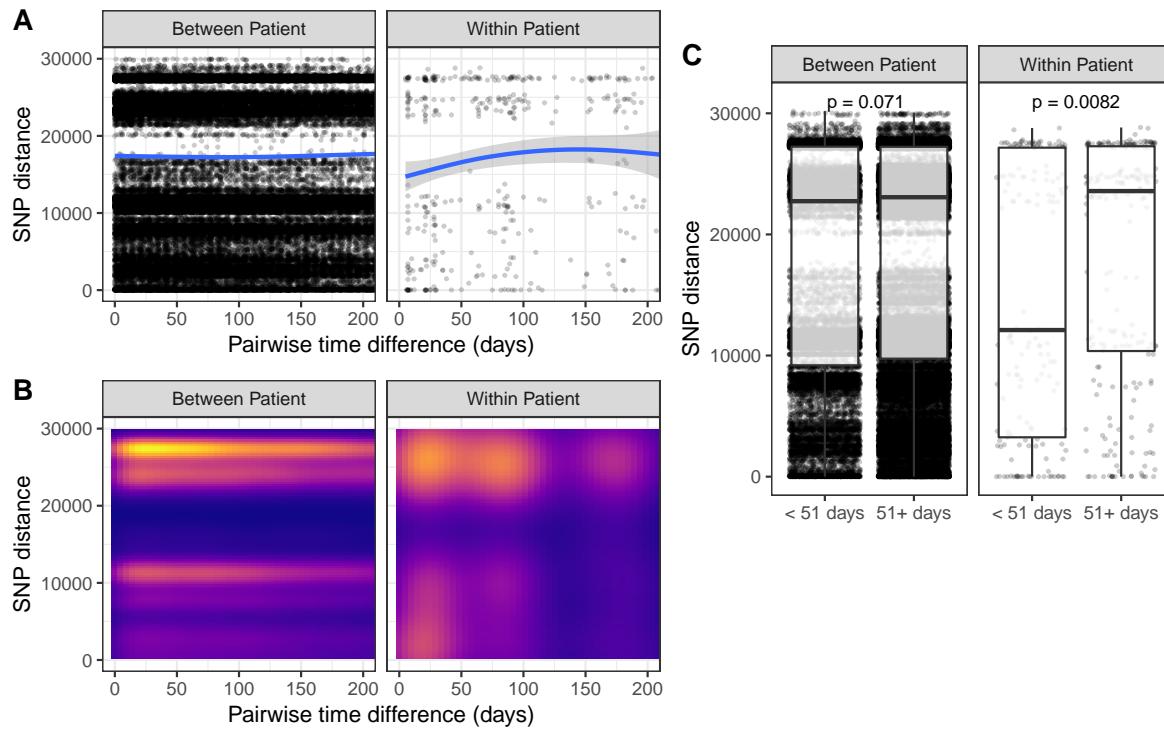


Figure 7.15: Within and between participant pairwise SNP distances. A: Scatterplot of pairwise SNP distances as a function of time with GAM model fitted curve. B: Pairwise SNP distance as function of time as a 2D density plot, showing cluster of isolates close to origin that are close together in time and SNP-distance. C: Pairwsise SNP distance distribution before and after 50 days, within and between patients, showing statistically significant descreas ein pairwise SNP distance within patients before 50 days. After 50 days, between- and within- patient distributuions are similar.

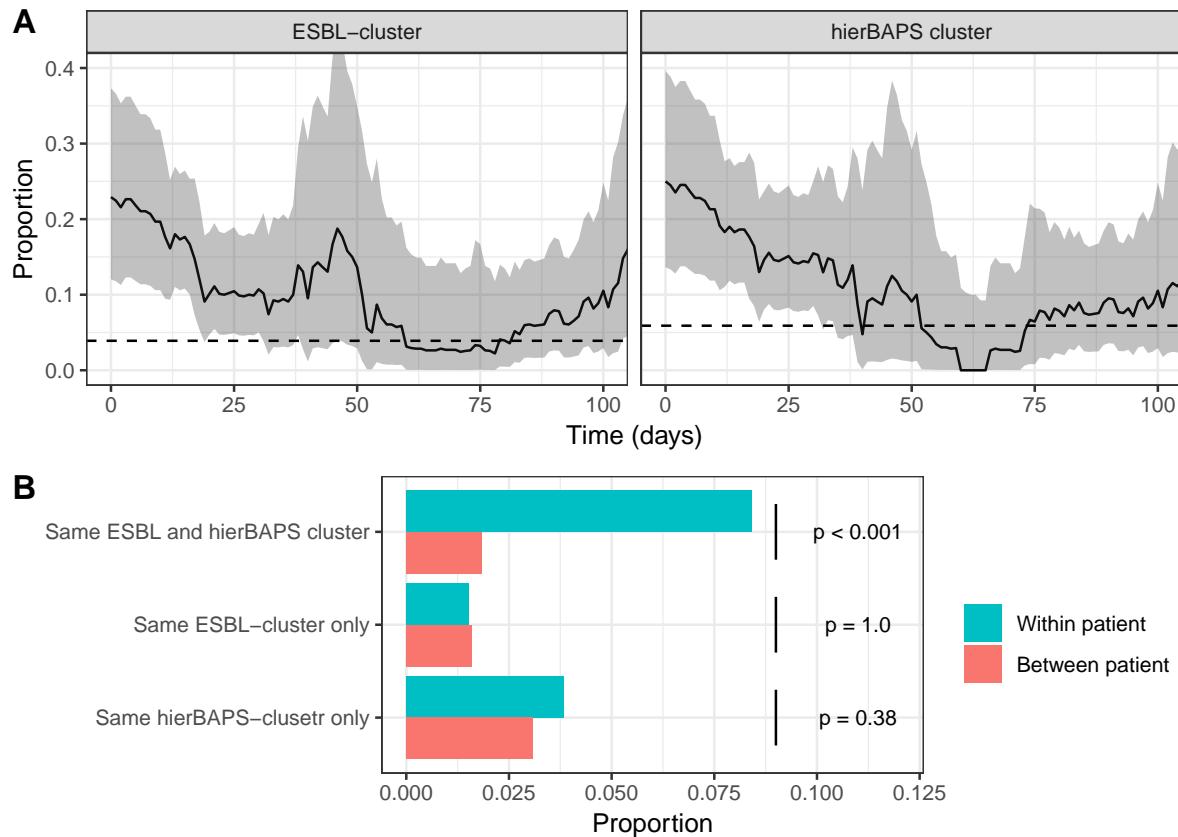


Figure 7.16: Probability of any two samples from within a given participant containing the same ESBL-cluster (A, left panel) or being a member of the same hierBAPS cluster (A, right panel). Time is windowed at ± 5 days around the time indicated on the x axis. Dotted line is the probability that two samples would belong to the same group by chance, constructed by randomly sampling 1000 sample pairs. B: proportion of samples that contain the same herBAPS cluster alone, or ESBL-cluster alone, or both, demonstrating that the ESBL cluster-hierBAPS cluster pairing is the most conserved of the three.

below discuss first the genomic landscape of the isolates in general terms, followed by insights that this analysis provides into the drivers of ESBL-E carriage.

7.4.1 Genomic landscape of ESBL *E. coli* in Malawi: global diversity and high-risk clones

The *E. coli* recovered from stool of the study participants in this study are diverse, encompassing the spectrum of diversity of the species with all major phylogroups and 56 STs represented. Phylogroup A was the commonest phylogroup seen, consistent with the traditional view of this phylogroup as associated with commensal strains[276]. When placed into a global phylogeny with context genomes from throughout the world, the Malawian isolates are largely distributed throughout the phylogeny: in a global context, Malawi is sampling the worldwide diversity of *E. coli*. The commonest ST in this study was the globally successful high risk clone ST131, mirroring the situation worldwide where this ST is thought to account for 40-80% of invasive ESBL *E. coli* infection[242,282].

There were, however, several areas of the global phylogeny where the Malawian isolates clustered tightly together, perhaps initially suggestive of Malawi-restricted clones; in considering the significance of this tree topology it is important to be cognisant of the biases inherent in the global *E. coli* collection, however. ESBL-producing *E. coli* are unusual in the ETEC[273] and GEMS[274] collections and all samples in these two studies were collected before 2011, though both of these collections are exclusively recovered from stool. In contrast, the clinical isolates from the Thai study[272] are all invasive, from a single centre, are selected on the basis of being ESBL-producers, and were isolated in 2014 or 2015. The isolates from the previous Malawian study were largely invasive[275], were selected for diversity in AMR profile, and were all isolated before 2014. There was no study that selectively cultured for ESBL producing *E. coli* in stool, as this study has done; in that, all of these studies are slightly sub-optimal for comparison. It may be that these biases contribute to apparent polyphyletic clustering of isolates from the current study in phylogroup A. It would be expected that ESBL producing phylogroup A *E. coli* would be underrepresented in the global collection compared to this study, as this phylogroup is associated with commensal (and hence stool) associated strains, and the two studies performing stool culture did not enrich for ESBL producers; the only study to do this collected invasive isolates, which may be expected to lie in phylogroup B2 over A.

Nevertheless, two of the three commonest STs identified in this study, ST410 and ST167, are unusual or absent in the global collection and could be considered to have a single introduction into Malawi in the context of the topology of the inferred phylogenetic tree. These could represent Malawi-restricted clades or, more likely given the diversity otherwise seen in the tree,

clades that are not represented in the global collection because of biases in sample selection. ST410 is recognised as an emerging high-risk clone which has been with isolated worldwide with some regularity since 2011 (including in Tanzania) and is associated with *bla_{CTXM-15}* and *bla_{NDM-5}*; coalescence analysis suggested a most recent common ancestor of ST410 of the early 1800s (similar to ST131[243]), and acquisition of *bla_{CTXM-15}* on a multireplicon IncFII-IncFIA-IncFIB plasmid in the late 1980s[283]. Similarly, ST167 has been recognised as commonly carrying ESBL genes and carbapenemases in Chinese invasive isolates[284] - indeed, it was the commonest *E. coli* ST in one longitudinal surveillance study of carbapenemases in 2012-16 in 25 Chinese provinces[285] - as well as being very prevalent among meat-associated *E. coli* in Germany between 2011-2013 in one study[286]. As such it, too, is also likely a successful global AMR-associated lineage.

It is therefore likely, especially given that these STs did not appear in the previous Malawian *E. coli* collection (in which all samples were collected prior to 2014), that they have recently arrived in Malawi. Alternatively, given that the previous Malawian collection was largely invasive, it could be that these STs do not often cause invasive disease, but the frequent identification of invasive ST410 and ST167 in the literature argues against this hypothesis, and it is more likely that they are recently arrived potential high-risk clones. If this is the case, then ST410 and 167 have become rapidly established in Blantyre over the course of only 2-3 years; in fact, this is exactly what was seen in longitudinal nationwide genomic surveillance of *E. coli* in the UK in 2003-04 when ST131 first arrived[287]. ST131 is now a well-established globally disseminated high-risk clone and the topography of the global tree suggests relatively unrestricted mixing between Malawian and global ST131. It may be that unbiased global sampling would reveal the same pattern for ST167 and ST410, and that they have also become globally prevalent; alternatively they may be truly geographically restricted either because they are in the process of spreading worldwide, or because some unknown factors prevent global spread. It is impossible to say from these data as they stand. Though some progress has been made in understanding the genomics of the emergence of ST131[243], the factors that contribute to its apparent fitness are unknown: it is impossible to predict, at present, from the genome of ST167 and ST410 whether they will repeat the course of ST131 to become truly globally dominant as a cause of human disease. Such an understanding of the determinants of fitness would be of great benefit in predicting and preventing global AMR spread.

7.4.2 Antimicrobial resistance determinants: domination of *bla_{CTXM-15}* and emergence of carbapenemases

The 473 isolates contained a diverse selection of antimicrobial resistance determinants, most with genotypic multiclass resistance. Genotypic and phenotypic co-trimoxazole resistance was near universal, as might be expected from a setting where lifelong co-trimoxazole preventative therapy (CPT) is mandated by the Malawian HIV treatment guidelines for HIV all infected adults[288], and mediated by *dfrA* and *Sul* alleles. Determinants of quinolone resistance were commonly identified, more frequently mutations in the QRDR than plasmid-mediated; chloramphenicol resistance genes were also common, most often *catB*. However, both of these correlated only moderately with phenotypic resistance as determined by disc diffusion testing. This is unexpected, as presence of chloramphenicol resistance genes has been shown to correlate well with phenotypic resistance[289], including in a study of 94 Malawian invasive isolates[290], though in this Malawian collection (the same collection of isolates as were included in the global collection in the current study), *catB* genes were rarely seen. Similarly, the QRDR GyrA83–ParC80–GyrA87 combination was found to be strongly correlated with quinolone resistance in a study of 10099 *E. coli* genomes[278]. There are several potential explanations for these discrepancies; the first is that antimicrobial sensitivity testing (AST) has incorrectly classified sensitive and resistance isolates. The AST method used was disc diffusion; certainly this is less accurate than an MIC determination method such as E-tests or dilution methods, and a true comparison of genotypic to phenotypic resistance - not the aim of this study - should use one of these methods. It is also possible that there were technical problems with the AST procedure (e.g. an overly heavy inoculum) though every attempt was made to avoid this, including with internal QC, and the work was carried out in a laboratory which subscribes to the UK NEQUAS QC procedure.

Alternatively, a gene may be present but not expressed, or expressed at a low level. This can not be the case for the point mutations in the QRDR conferring quinolone resistance, but is possible for *catB4*, which was commonly present in phenotypically chloramphenicol sensitive isolates. Interestingly, truncated *catB4* elements (often in conjunction with an IS26 transposon) were almost universal in the isolates in this study: of 233 isolates in which *ARIBA* did not assemble a full *catB4* sequence, 226/233 (97%) contained a truncated *catB4* element. This configuration (*catB4* truncated by an IS26 element) has been described in Enterobacteriaceae[[291]; Sekizuka2018]. It could certainly be unrelated but its ubiquity in this study raises at least the possibility of misassembly and false-positive identification of *catB4* in some cases. Long read sequencing would allow description of the genetic environment of *catB4* in this collection and an understanding of its expression, which might allow better correlation with phenotype.

ESBL resistance in this collection is dominated by *bla_{CTXM}* and *bla_{CTXM-15}* in particular; this latter gene was carried by 319/473 [67%] of isolates. The only non-*bla_{CTXM}* ESBL gene identified in any significant prevalence was *bla_{SHV-12}*; ESBL *bla_{OXA}* and *bla_{TEM}* were very rare, though narrow-spectrum penicillinase alleles of this family were common. The dominance of *bla_{CTXM-15}* is in keeping with the situation seen worldwide[238]. In this collection, *bla_{CTXM-15}* was spread throughout the phylogeny rather than associated with any particular clade. I identified one carbapenemase, *bla_{NDM-5}*, carried on a globally successful IncX3 plasmid. To my knowledge, this is the first carbapenemase to be described in Malawi. Carbapenem antimicrobials were introduced to the Malawian essential medicines list in 2015 but are at best sporadically available, and only in tertiary centres, often for truncated courses. The emergence of carbapenemases with such minimal carbapenem use and so soon after introduction is troubling, and should prompt discussions regarding the best use of this precious antimicrobial class; certainly, given the high prevalence of ESBL production among invasive *Klebsiella pneumoniae* and *E. coli*[246], there is a case for expanded access but optimal antimicrobial stewardship strategies in this context are unknown.

7.4.3 Drivers of ESBL-E carriage: true acquisition versus enrichment

The diversity of healthcare associated isolates was largely contained within the diversity of community isolates, rather than apparent hospital acquisitions being restricted to a single clade or clone. The exception to this was SC23, which corresponded to ST410, and was more likely to be healthcare associated. This could be consistent with the hypothesis that it is a recently arrived high-risk clone, which may be, at least initially, hospital-associated. Even so, it is clearly not hospital restricted, with half of the ST410 isolates being isolated from the community. This result could be explained by two hypotheses: first, that these are true transmission events that are occurring within the hospital, and that the diversity of ESBL *E. coli* within the hospital is the same as the community; or, second, that these “hospital acquisitions” are actually minority variant *E. coli* present in the microbiota (and therefore acquired in the community) at a low abundance and hence not detected by culture, and enriched for with antimicrobial exposure in hospital. This latter hypothesis is perhaps supported by the fact that the antimicrobial-exposed group of patients in this study have an early and rapid increase in ESBL-E carriage prevalence that is not seen in the antimicrobial-unexposed group. Distinguishing between these two hypotheses is important as they would each require a different intervention: hospital infection control in the former case, or strategies to protect the microbiota from the deleterious effect of broad spectrum antimicrobials (such as pre- or probiotics, or oral β -lactamases) in the latter.

By forming sequence clusters and ESBL-clusters, I was able to demonstrate that both bacteria

and MGE are conserved together, within-patient, over time. Some previous longitudinal studies of ESBL-E found that *E. coli* STs tended to vary over time but that in many cases ESBL gene and plasmid replicon remained the same, which could be due to a conserved MGE transferring between bacteria[231]. Given my findings, this is unlikely to be the case. Though not directly addressed in this study it is possible to speculate therefore that the unit of transmission of ESBL between patients is likely to be the bacterium rather than, for example, horizontal gene transfer of ESBL genes on plasmids or transposons. The within-patient correlation of SC and ESBL-cluster lasts only for 32-35 days; two samples from a single patient more than 35 days apart are as likely to contain the same SC/ESBL cluster as two samples from two different patients. This implies either an exogenous re-exposure or some other endogenous mechanism whereby the dominant ESBL strain is replaced by a minority variant from within the microbiota. Of note, the timescale of SC replacement - occurring after 35 days - is consistent with the mean time taken to revert to the ESBL-negative state from ESBL-positive from the longitudinal Markov models (26 [95% CI 12-58] days), perhaps lending support to the re-exposure hypothesis. It is important to note also that even at a maximum (i.e. with samples that are days apart), only around 20% of within-patient samples contain the same SC/ESBL-cluster. This is many times more than would be expected by chance, but still implies that at any time point there is significant diversity of ESBL *E. coli* strains, that have been missed by only taking forward one colony pick for sequencing. This is consistent with studies that have performed multiple colony picks on stool samples enriched for ESBL-E, and have found widespread diversity of STs and ESBL genes[292].

7.4.4 Study limitations

There are several limitations to the analysis carried out in this chapter. The most serious is due to the fact that only one colony pick from the ESBL selective media was taken forward for sequencing. This was inevitable because of resource considerations, but it is clear that there is significant within-patient unsampled diversity as a result. In effect, we have randomly sampled one strain from all available strains at any give time point. This is likely to result in an underestimation of the extent to which strains persist within the individual over time, as strains that are present (but not sampled) are classed as absent. I have focused on *E. coli*; in fact, these were often isolated in conjunction with other bacteria, most notably *Klebsiella pneumoniae*, which were not included in this analysis, so the problem of unsampled diversity is in fact even more severe. The diversity of ESBL genes in Blantyre is likely therefore to be greater than I describe.

I selected a global collection of *E. coli* based on what was available but, as described above and in common with many analyses of this type, this is a biased collection. This must be

borne in mind when interpreting the global phylogeny. There are inherent limitations in the short-read Illumina sequencing that was carried out: assembly of areas with multiple nucleotide repeats (as found in plasmids and transposable elements in particular) is difficult or impossible, making it impossible to fully characterise the MGE in this dataset upon which the AMR genes are carried. I have attempted to address this difficulty by defining ESBL-clusters as a proxy for MGE, but this is by its nature flawed. Some of the assembled contigs are short and likely represent transposons; the same transposons have likely inserted into multiple plasmids in the past and as such, these short contigs may cluster with other sequences that would be seen to be very different, were a full assembly available. In addition, the biological significance of these ESBL-clusters is not clear. It is not possible to say with certainty what they represent (e.g. plasmid) as they are only fragments. Nevertheless, the fact that I have seen within-patient associations of the ESBL-clusters lends some support to their use, as erroneous clustering would be expected to bias any associations towards to null.

7.4.5 Conclusions and further work

In conclusion, I have shown that the *E. coli* population in this study is diverse, representing global *E. coli* diversity; on a smaller scale, the diversity of healthcare-associated isolates is represented in the community. These facts together suggest widespread mixing of strains at multiple spatial levels. It is possible that apparent constant colonisation actually represents frequent re-exposure on the timescale of around 35 days, and that apparent hospital acquisitions, are, in fact, unmasking events due to enrichment of the microbiota for ESBL-E by antimicrobial exposure.

Many questions remain unanswered and further work is necessary. Shotgun metagenomic sequencing of stool would allow testing of the competing acquisition and unmasking hypotheses of rapid increase in ESBL-E prevalence by defining the microbiota and total AMR gene content pre-, during and post- antimicrobial exposure, as well as providing an opportunity to explore the role of the microbiota to colonisation resistance to ESBL-E. Long read sequencing would allow a proper characterisation of the MGE that carry ESBL genes in the Malawian context, giving the resolution necessary to truly track MGE within and between patients and strains, as well as to address questions of expression of genes such as *catB4* that seem to correlate poorly with phenotype, by examining e.g. promoter regions. Short-read sequencing of the *Klebsiella pneumoniae* isolates from this study would allow a comparison between the mechanisms of AMR and MGE prevalent in this species as compared to *E. coli*, and assess the extent to which horizontal gene transfer between the two is driving ESBL spread in Blantyre. Collating publicly available ST167 and ST410 genomes would allow the construction of a phylogeny that would allow insight into the epidemiology of these putative recently arrived high risk

clones. Finally, incorporating the resolution afforded by sequencing into the longitudinal Markov models may provide new insights into the dynamics of ESBL-E carriage. This is the subject of the next chapter.

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
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7.5 Appendix

Table 7.1: Details of samples included in global phylogeny

Study	Sample ID	Accession No.	Source	Country
This study	26141_1_134		Stool	Malawi
This study	26141_1_135		Stool	Malawi
This study	26141_1_136		Stool	Malawi
This study	26141_1_137		Stool	Malawi
This study	26141_1_138		Stool	Malawi
This study	26141_1_139		Stool	Malawi
This study	26141_1_140		Stool	Malawi
This study	26141_1_141		Stool	Malawi
This study	26141_1_142		Stool	Malawi
This study	26141_1_143		Stool	Malawi
This study	26141_1_144		Stool	Malawi
This study	26141_1_145		Stool	Malawi
This study	26141_1_146		Stool	Malawi
This study	26141_1_147		Stool	Malawi
This study	26141_1_148		Stool	Malawi
This study	26141_1_149		Stool	Malawi
This study	26141_1_150		Stool	Malawi
This study	26141_1_151		Stool	Malawi
This study	26141_1_152		Stool	Malawi
This study	26141_1_153		Stool	Malawi
This study	26141_1_154		Stool	Malawi
This study	26141_1_155		Stool	Malawi
This study	26141_1_156		Stool	Malawi
This study	26141_1_157		Stool	Malawi
This study	26141_1_158		Stool	Malawi

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
This study	26141_1_159		Stool	Malawi
This study	26141_1_160		Stool	Malawi
This study	26141_1_161		Stool	Malawi
This study	26141_1_162		Stool	Malawi
This study	26141_1_164		Stool	Malawi
This study	26141_1_165		Stool	Malawi
This study	26141_1_166		Stool	Malawi
This study	26141_1_168		Stool	Malawi
This study	26141_1_169		Stool	Malawi
This study	26141_1_170		Stool	Malawi
This study	26141_1_171		Stool	Malawi
This study	26141_1_172		Stool	Malawi
This study	26141_1_173		Stool	Malawi
This study	26141_1_174		Stool	Malawi
This study	26141_1_175		Stool	Malawi
This study	26141_1_176		Stool	Malawi
This study	26141_1_177		Stool	Malawi
This study	26141_1_178		Stool	Malawi
This study	26141_1_179		Stool	Malawi
This study	26141_1_180		Stool	Malawi
This study	26141_1_181		Stool	Malawi
This study	26141_1_182		Stool	Malawi
This study	26141_1_183		Stool	Malawi
This study	26141_1_184		Stool	Malawi
This study	26141_1_186		Stool	Malawi
This study	26141_1_187		Stool	Malawi
This study	26141_1_189		Stool	Malawi
This study	26141_1_190		Stool	Malawi
This study	26141_1_191		Stool	Malawi
This study	26141_1_192		Stool	Malawi
This study	26141_1_193		Stool	Malawi

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
This study	26141_1_194		Stool	Malawi
This study	26141_1_195		Stool	Malawi
This study	26141_1_196		Stool	Malawi
This study	26141_1_197		Stool	Malawi
This study	26141_1_198		Stool	Malawi
This study	26141_1_199		Stool	Malawi
This study	26141_1_200		Stool	Malawi
This study	26141_1_201		Stool	Malawi
This study	26141_1_202		Stool	Malawi
This study	26141_1_203		Stool	Malawi
This study	26141_1_204		Stool	Malawi
This study	26141_1_205		Stool	Malawi
This study	26141_1_206		Stool	Malawi
This study	26141_1_207		Stool	Malawi
This study	26141_1_208		Stool	Malawi
This study	26141_1_209		Stool	Malawi
This study	26141_1_210		Stool	Malawi
This study	26141_1_211		Stool	Malawi
This study	26141_1_212		Stool	Malawi
This study	26141_1_213		Stool	Malawi
This study	26141_1_214		Stool	Malawi
This study	26141_1_215		Stool	Malawi
This study	26141_1_217		Stool	Malawi
This study	26141_1_218		Stool	Malawi
This study	26141_1_219		Stool	Malawi
This study	26141_1_220		Stool	Malawi
This study	26141_1_221		Stool	Malawi
This study	26141_1_222		Stool	Malawi
This study	26141_1_223		Stool	Malawi
This study	26141_1_224		Stool	Malawi
This study	26141_1_225		Stool	Malawi
This study	26141_1_226		Stool	Malawi

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
This study	26141_1_227		Stool	Malawi
This study	26141_1_228		Stool	Malawi
This study	26141_1_229		Stool	Malawi
This study	26141_1_230		Stool	Malawi
This study	26141_1_232		Stool	Malawi
This study	26141_1_236		Stool	Malawi
This study	26141_1_237		Stool	Malawi
This study	26141_1_239		Stool	Malawi
This study	26141_1_240		Stool	Malawi
This study	26141_1_241		Stool	Malawi
This study	26141_1_242		Stool	Malawi
This study	26141_1_243		Stool	Malawi
This study	26141_1_244		Stool	Malawi
This study	26141_1_246		Stool	Malawi
This study	26141_1_247		Stool	Malawi
This study	26141_1_248		Stool	Malawi
This study	26141_1_250		Stool	Malawi
This study	26141_1_251		Stool	Malawi
This study	26141_1_252		Stool	Malawi
This study	26141_1_253		Stool	Malawi
This study	26141_1_254		Stool	Malawi
This study	26141_1_255		Stool	Malawi
This study	26141_1_256		Stool	Malawi
This study	26141_1_257		Stool	Malawi
This study	26141_1_258		Stool	Malawi
This study	26141_1_259		Stool	Malawi
This study	26141_1_260		Stool	Malawi
This study	26141_1_261		Stool	Malawi
This study	26141_1_262		Stool	Malawi
This study	26141_1_263		Stool	Malawi
This study	26141_1_265		Stool	Malawi
This study	26141_1_266		Stool	Malawi

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
This study	26141_1_267		Stool	Malawi
This study	26141_1_268		Stool	Malawi
This study	26141_1_270		Stool	Malawi
This study	26141_1_271		Stool	Malawi
This study	26141_1_272		Stool	Malawi
This study	26141_1_273		Stool	Malawi
This study	26141_1_274		Stool	Malawi
This study	26141_1_275		Stool	Malawi
This study	26141_1_276		Stool	Malawi
This study	26141_1_277		Stool	Malawi
This study	26141_1_278		Stool	Malawi
This study	26141_1_279		Stool	Malawi
This study	26141_1_280		Stool	Malawi
This study	26141_1_282		Stool	Malawi
This study	26141_1_283		Stool	Malawi
This study	26141_1_284	ERR3168700	Stool	Malawi
This study	26141_1_285		Stool	Malawi
This study	26141_1_286		Stool	Malawi
This study	26141_1_287		Stool	Malawi
This study	26141_1_288		Stool	Malawi
This study	26141_1_289		Stool	Malawi
This study	26141_1_290		Stool	Malawi
This study	26141_1_291		Stool	Malawi
This study	26141_1_292		Stool	Malawi
This study	26141_1_293		Stool	Malawi
This study	26141_1_295		Stool	Malawi
This study	26141_1_296		Stool	Malawi
This study	26141_1_297		Stool	Malawi
This study	26141_1_298		Stool	Malawi
This study	26141_1_299		Stool	Malawi
This study	28099_1_1		Stool	Malawi

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
This study	28099_1_10		Stool	Malawi
This study	28099_1_100		Stool	Malawi
This study	28099_1_102		Stool	Malawi
This study	28099_1_103		Stool	Malawi
This study	28099_1_104		Stool	Malawi
This study	28099_1_106		Stool	Malawi
This study	28099_1_107		Stool	Malawi
This study	28099_1_11		Stool	Malawi
This study	28099_1_110		Stool	Malawi
This study	28099_1_111		Stool	Malawi
This study	28099_1_112		Stool	Malawi
This study	28099_1_114		Stool	Malawi
This study	28099_1_115		Stool	Malawi
This study	28099_1_116		Stool	Malawi
This study	28099_1_118		Stool	Malawi
This study	28099_1_119		Stool	Malawi
This study	28099_1_120		Stool	Malawi
This study	28099_1_123		Stool	Malawi
This study	28099_1_125		Stool	Malawi
This study	28099_1_127		Stool	Malawi
This study	28099_1_128		Stool	Malawi
This study	28099_1_129		Stool	Malawi
This study	28099_1_131		Stool	Malawi
This study	28099_1_132		Stool	Malawi
This study	28099_1_133		Stool	Malawi
This study	28099_1_135		Stool	Malawi
This study	28099_1_136		Stool	Malawi
This study	28099_1_137		Stool	Malawi
This study	28099_1_139		Stool	Malawi
This study	28099_1_14		Stool	Malawi
This study	28099_1_141		Stool	Malawi
This study	28099_1_143		Stool	Malawi

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
This study	28099_1_144		Stool	Malawi
This study	28099_1_145		Stool	Malawi
This study	28099_1_148		Stool	Malawi
This study	28099_1_149		Stool	Malawi
This study	28099_1_151		Stool	Malawi
This study	28099_1_152		Stool	Malawi
This study	28099_1_153		Stool	Malawi
This study	28099_1_155		Stool	Malawi
This study	28099_1_156		Stool	Malawi
This study	28099_1_157		Stool	Malawi
This study	28099_1_159		Stool	Malawi
This study	28099_1_160		Stool	Malawi
This study	28099_1_161		Stool	Malawi
This study	28099_1_163		Stool	Malawi
This study	28099_1_165		Stool	Malawi
This study	28099_1_167		Stool	Malawi
This study	28099_1_168		Stool	Malawi
This study	28099_1_169		Stool	Malawi
This study	28099_1_171		Stool	Malawi
This study	28099_1_172		Stool	Malawi
This study	28099_1_173		Stool	Malawi
This study	28099_1_175		Stool	Malawi
This study	28099_1_176		Stool	Malawi
This study	28099_1_177		Stool	Malawi
This study	28099_1_179		Stool	Malawi
This study	28099_1_18		Stool	Malawi
This study	28099_1_180		Stool	Malawi
This study	28099_1_181		Stool	Malawi
This study	28099_1_185		Stool	Malawi
This study	28099_1_187		Stool	Malawi
This study	28099_1_188		Stool	Malawi
This study	28099_1_189		Stool	Malawi

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
This study	28099_1_19		Stool	Malawi
This study	28099_1_191		Stool	Malawi
This study	28099_1_192		Stool	Malawi
This study	28099_1_193		Stool	Malawi
This study	28099_1_195		Stool	Malawi
This study	28099_1_196		Stool	Malawi
This study	28099_1_199		Stool	Malawi
This study	28099_1_2		Stool	Malawi
This study	28099_1_200		Stool	Malawi
This study	28099_1_203		Stool	Malawi
This study	28099_1_204		Stool	Malawi
This study	28099_1_205		Stool	Malawi
This study	28099_1_207		Stool	Malawi
This study	28099_1_208		Stool	Malawi
This study	28099_1_209		Stool	Malawi
This study	28099_1_211		Stool	Malawi
This study	28099_1_212		Stool	Malawi
This study	28099_1_213		Stool	Malawi
This study	28099_1_214		Stool	Malawi
This study	28099_1_216		Stool	Malawi
This study	28099_1_217		Stool	Malawi
This study	28099_1_218		Stool	Malawi
This study	28099_1_22		Stool	Malawi
This study	28099_1_220		Stool	Malawi
This study	28099_1_221		Stool	Malawi
This study	28099_1_222		Stool	Malawi
This study	28099_1_224		Stool	Malawi
This study	28099_1_225		Stool	Malawi
This study	28099_1_226		Stool	Malawi
This study	28099_1_228		Stool	Malawi
This study	28099_1_229		Stool	Malawi

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
This study	28099_1_23		Stool	Malawi
This study	28099_1_230		Stool	Malawi
This study	28099_1_232		Stool	Malawi
This study	28099_1_233		Stool	Malawi
This study	28099_1_234		Stool	Malawi
This study	28099_1_236		Stool	Malawi
This study	28099_1_237		Stool	Malawi
This study	28099_1_238		Stool	Malawi
This study	28099_1_240		Stool	Malawi
This study	28099_1_241		Stool	Malawi
This study	28099_1_242		Stool	Malawi
This study	28099_1_244		Stool	Malawi
This study	28099_1_245		Stool	Malawi
This study	28099_1_246		Stool	Malawi
This study	28099_1_248		Stool	Malawi
This study	28099_1_249		Stool	Malawi
This study	28099_1_250		Stool	Malawi
This study	28099_1_252		Stool	Malawi
This study	28099_1_253		Stool	Malawi
This study	28099_1_254		Stool	Malawi
This study	28099_1_256		Stool	Malawi
This study	28099_1_257		Stool	Malawi
This study	28099_1_258		Stool	Malawi
This study	28099_1_26		Stool	Malawi
This study	28099_1_260		Stool	Malawi
This study	28099_1_261		Stool	Malawi
This study	28099_1_264		Stool	Malawi
This study	28099_1_266		Stool	Malawi
This study	28099_1_268		Stool	Malawi
This study	28099_1_269		Stool	Malawi
This study	28099_1_27		Stool	Malawi
This study	28099_1_270		Stool	Malawi

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
This study	28099_1_272		Stool	Malawi
This study	28099_1_273		Stool	Malawi
This study	28099_1_274		Stool	Malawi
This study	28099_1_277		Stool	Malawi
This study	28099_1_278		Stool	Malawi
This study	28099_1_280		Stool	Malawi
This study	28099_1_281		Stool	Malawi
This study	28099_1_282		Stool	Malawi
This study	28099_1_284		Stool	Malawi
This study	28099_1_285		Stool	Malawi
This study	28099_1_286		Stool	Malawi
This study	28099_1_288		Stool	Malawi
This study	28099_1_289		Stool	Malawi
This study	28099_1_293		Stool	Malawi
This study	28099_1_294		Stool	Malawi
This study	28099_1_297		Stool	Malawi
This study	28099_1_30		Stool	Malawi
This study	28099_1_300		Stool	Malawi
This study	28099_1_301		Stool	Malawi
This study	28099_1_302		Stool	Malawi
This study	28099_1_303		Stool	Malawi
This study	28099_1_305		Stool	Malawi
This study	28099_1_306		Stool	Malawi
This study	28099_1_307		Stool	Malawi
This study	28099_1_309		Stool	Malawi
This study	28099_1_31		Stool	Malawi
This study	28099_1_311		Stool	Malawi
This study	28099_1_313		Stool	Malawi
This study	28099_1_314		Stool	Malawi
This study	28099_1_315		Stool	Malawi
This study	28099_1_317		Stool	Malawi
This study	28099_1_318		Stool	Malawi

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
This study	28099_1_319		Stool	Malawi
This study	28099_1_321		Stool	Malawi
This study	28099_1_322		Stool	Malawi
This study	28099_1_323		Stool	Malawi
This study	28099_1_325		Stool	Malawi
This study	28099_1_326		Stool	Malawi
This study	28099_1_327		Stool	Malawi
This study	28099_1_329		Stool	Malawi
This study	28099_1_330		Stool	Malawi
This study	28099_1_331		Stool	Malawi
This study	28099_1_333		Stool	Malawi
This study	28099_1_334		Stool	Malawi
This study	28099_1_335		Stool	Malawi
This study	28099_1_337		Stool	Malawi
This study	28099_1_338		Stool	Malawi
This study	28099_1_339		Stool	Malawi
This study	28099_1_34		Stool	Malawi
This study	28099_1_341		Stool	Malawi
This study	28099_1_342		Stool	Malawi
This study	28099_1_343		Stool	Malawi
This study	28099_1_345		Stool	Malawi
This study	28099_1_346		Stool	Malawi
This study	28099_1_347		Stool	Malawi
This study	28099_1_349		Stool	Malawi
This study	28099_1_35		Stool	Malawi
This study	28099_1_350		Stool	Malawi
This study	28099_1_351		Stool	Malawi
This study	28099_1_353		Stool	Malawi
This study	28099_1_354		Stool	Malawi
This study	28099_1_355		Stool	Malawi
This study	28099_1_357		Stool	Malawi

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
This study	28099_1_358		Stool	Malawi
This study	28099_1_359		Stool	Malawi
This study	28099_1_361		Stool	Malawi
This study	28099_1_362		Stool	Malawi
This study	28099_1_363		Stool	Malawi
This study	28099_1_365		Stool	Malawi
This study	28099_1_366		Stool	Malawi
This study	28099_1_367		Stool	Malawi
This study	28099_1_370		Stool	Malawi
This study	28099_1_371		Stool	Malawi
This study	28099_1_373		Stool	Malawi
This study	28099_1_374		Stool	Malawi
This study	28099_1_375		Stool	Malawi
This study	28099_1_377		Stool	Malawi
This study	28099_1_378		Stool	Malawi
This study	28099_1_379		Stool	Malawi
This study	28099_1_38		Stool	Malawi
This study	28099_1_381		Stool	Malawi
This study	28099_1_382		Stool	Malawi
This study	28099_1_383		Stool	Malawi
This study	28099_1_39		Stool	Malawi
This study	28099_1_41		Stool	Malawi
This study	28099_1_42		Stool	Malawi
This study	28099_1_43		Stool	Malawi
This study	28099_1_46		Stool	Malawi
This study	28099_1_47		Stool	Malawi
This study	28099_1_49		Stool	Malawi
This study	28099_1_50		Stool	Malawi
This study	28099_1_51		Stool	Malawi
This study	28099_1_53		Stool	Malawi
This study	28099_1_54		Stool	Malawi
This study	28099_1_55		Stool	Malawi

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
This study	28099_1_57		Stool	Malawi
This study	28099_1_58		Stool	Malawi
This study	28099_1_59		Stool	Malawi
This study	28099_1_61		Stool	Malawi
This study	28099_1_62		Stool	Malawi
This study	28099_1_63		Stool	Malawi
This study	28099_1_65		Stool	Malawi
This study	28099_1_66		Stool	Malawi
This study	28099_1_69		Stool	Malawi
This study	28099_1_7		Stool	Malawi
This study	28099_1_70		Stool	Malawi
This study	28099_1_71		Stool	Malawi
This study	28099_1_73		Stool	Malawi
This study	28099_1_74		Stool	Malawi
This study	28099_1_75		Stool	Malawi
This study	28099_1_77		Stool	Malawi
This study	28099_1_78		Stool	Malawi
This study	28099_1_79		Stool	Malawi
This study	28099_1_81		Stool	Malawi
This study	28099_1_82		Stool	Malawi
This study	28099_1_83		Stool	Malawi
This study	28099_1_85		Stool	Malawi
This study	28099_1_86		Stool	Malawi
This study	28099_1_87		Stool	Malawi
This study	28099_1_89		Stool	Malawi
This study	28099_1_90		Stool	Malawi
This study	28099_1_91		Stool	Malawi
This study	28099_1_93		Stool	Malawi
This study	28099_1_94		Stool	Malawi
This study	28099_1_95		Stool	Malawi
This study	28099_1_98		Stool	Malawi
This study	28099_1_99		Stool	Malawi

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
This study	28099_2_101		Stool	Malawi
This study	28099_2_105		Stool	Malawi
This study	28099_2_109		Stool	Malawi
This study	28099_2_113		Stool	Malawi
This study	28099_2_117		Stool	Malawi
This study	28099_2_12		Stool	Malawi
This study	28099_2_121		Stool	Malawi
This study	28099_2_126		Stool	Malawi
This study	28099_2_130		Stool	Malawi
This study	28099_2_138		Stool	Malawi
This study	28099_2_142		Stool	Malawi
This study	28099_2_146		Stool	Malawi
This study	28099_2_150		Stool	Malawi
This study	28099_2_154		Stool	Malawi
This study	28099_2_158		Stool	Malawi
This study	28099_2_16		Stool	Malawi
This study	28099_2_162		Stool	Malawi
This study	28099_2_166		Stool	Malawi
This study	28099_2_170		Stool	Malawi
This study	28099_2_174		Stool	Malawi
This study	28099_2_178		Stool	Malawi
This study	28099_2_182		Stool	Malawi
This study	28099_2_186		Stool	Malawi
This study	28099_2_190		Stool	Malawi
This study	28099_2_194		Stool	Malawi
This study	28099_2_198		Stool	Malawi
This study	28099_2_206		Stool	Malawi
This study	28099_2_210		Stool	Malawi
This study	28099_2_215		Stool	Malawi
This study	28099_2_219		Stool	Malawi
This study	28099_2_223		Stool	Malawi

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
This study	28099_2_227		Stool	Malawi
This study	28099_2_231		Stool	Malawi
This study	28099_2_235		Stool	Malawi
This study	28099_2_239		Stool	Malawi
This study	28099_2_24		Stool	Malawi
This study	28099_2_243		Stool	Malawi
This study	28099_2_247		Stool	Malawi
This study	28099_2_251		Stool	Malawi
This study	28099_2_255		Stool	Malawi
This study	28099_2_259		Stool	Malawi
This study	28099_2_263		Stool	Malawi
This study	28099_2_283		Stool	Malawi
This study	28099_2_287		Stool	Malawi
This study	28099_2_291		Stool	Malawi
This study	28099_2_295		Stool	Malawi
This study	28099_2_299		Stool	Malawi
This study	28099_2_3		Stool	Malawi
This study	28099_2_304		Stool	Malawi
This study	28099_2_308		Stool	Malawi
This study	28099_2_316		Stool	Malawi
This study	28099_2_32		Stool	Malawi
This study	28099_2_320		Stool	Malawi
This study	28099_2_36		Stool	Malawi
This study	28099_2_40		Stool	Malawi
This study	28099_2_44		Stool	Malawi
This study	28099_2_48		Stool	Malawi
This study	28099_2_56		Stool	Malawi
This study	28099_2_60		Stool	Malawi
This study	28099_2_64		Stool	Malawi
This study	28099_2_76		Stool	Malawi
This study	28099_2_8		Stool	Malawi
This study	28099_2_80		Stool	Malawi

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
This study	28099_2_84		Stool	Malawi
This study	28099_2_88		Stool	Malawi
This study	28099_2_92		Stool	Malawi
This study	28099_2_96		Stool	Malawi
This study	28099_2_97		Stool	Malawi
Ingle 2018	100269_aEPEC	ERR134513	Stool	Gambia
Ingle 2018	100383_aEPEC	ERR137807	Stool	Gambia
Ingle 2018	100446	ERR178176	Stool	Gambia
Ingle 2018	100554_aEPEC	ERR134514	Stool	Gambia
Ingle 2018	100600_aEPEC	ERR134515	Stool	Gambia
Ingle 2018	102010_aEPEC	ERR137808	Stool	Gambia
Ingle 2018	102014_aEPEC	ERR137809	Stool	Gambia
Ingle 2018	102298_aEPEC	ERR134516	Stool	Gambia
Ingle 2018	102328_aEPEC	ERR134517	Stool	Gambia
Ingle 2018	102366_aEPEC	ERR137810	Stool	Gambia
Ingle 2018	102485_aEPEC	ERR134518	Stool	Gambia
Ingle 2018	103151	ERR178192	Stool	Gambia
Ingle 2018	200135_aEPEC	ERR134519	Stool	Mali
Ingle 2018	200232	ERR178150	Stool	Mali
Ingle 2018	200439_aEPEC	ERR134520	Stool	Mali
Ingle 2018	200456_aEPEC	ERR137812	Stool	Mali
Ingle 2018	200499	ERR178148	Stool	Mali
Ingle 2018	200696	ERR178151	Stool	Mali
Ingle 2018	200708_aEPEC	ERR137782	Stool	Mali
Ingle 2018	200758_aEPEC	ERR137783	Stool	Mali
Ingle 2018	200781_aEPEC	ERR124658	Stool	Mali
Ingle 2018	200959_aEPEC	ERR137813	Stool	Mali
Ingle 2018	201191_aEPEC	ERR137814	Stool	Mali
Ingle 2018	201214_aEPEC	ERR134521	Stool	Mali
Ingle 2018	201350	ERR178216	Stool	Mali
Ingle 2018	201381_aEPEC	ERR137784	Stool	Mali
Ingle 2018	201488_aEPEC	ERR137815	Stool	Mali

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Ingle 2018	201534_aEPEC	ERR134522	Stool	Mali
Ingle 2018	201589_aEPEC	ERR137816	Stool	Mali
Ingle 2018	202317_aEPEC	ERR137817	Stool	Mali
Ingle 2018	202374	ERR178152	Stool	Mali
Ingle 2018	202387	ERR178149	Stool	Mali
Ingle 2018	202423_aEPEC	ERR134523	Stool	Mali
Ingle 2018	202443_aEPEC	ERR134524	Stool	Mali
Ingle 2018	202453_aEPEC	ERR134525	Stool	Mali
Ingle 2018	202474	ERR178153	Stool	Mali
Ingle 2018	202521_aEPEC	ERR124659	Stool	Mali
Ingle 2018	202621_aEPEC	ERR137818	Stool	Mali
Ingle 2018	202833_aEPEC	ERR134526	Stool	Mali
Ingle 2018	202973_aEPEC	ERR134527	Stool	Mali
Ingle 2018	203470_aEPEC	ERR124660	Stool	Mali
Ingle 2018	204263_aEPEC	ERR124661	Stool	Mali
Ingle 2018	204302_aEPEC	ERR134528	Stool	Mali
Ingle 2018	300073	ERR178193	Stool	Mozambique
Ingle 2018	300086_aEPEC	ERR134529	Stool	Mozambique
Ingle 2018	300711_aEPEC	ERR134530	Stool	Mozambique
Ingle 2018	300795_aEPEC	ERR134531	Stool	Mozambique
Ingle 2018	300812_aEPEC	ERR137819	Stool	Mozambique
Ingle 2018	300814_aEPEC	ERR137820	Stool	Mozambique
Ingle 2018	302082	ERR178198	Stool	Mozambique
Ingle 2018	302302	ERR178154	Stool	Mozambique
Ingle 2018	302613	ERR178210	Stool	Mozambique
Ingle 2018	302619	ERR178211	Stool	Mozambique
Ingle 2018	302700	ERR178217	Stool	Mozambique
Ingle 2018	302701	ERR178212	Stool	Mozambique
Ingle 2018	302710	ERR178218	Stool	Mozambique
Ingle 2018	400549_aEPEC	ERR137785	Stool	Kenya
Ingle 2018	400654_aEPEC	ERR137786	Stool	Kenya

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Ingle 2018	400714_aEPEC	ERR137787	Stool	Kenya
Ingle 2018	400896	ERR178177	Stool	Kenya
Ingle 2018	400897_aEPEC	ERR137821	Stool	Kenya
Ingle 2018	400998_aEPEC	ERR137789	Stool	Kenya
Ingle 2018	401082	ERR178178	Stool	Kenya
Ingle 2018	401117_aEPEC	ERR137822	Stool	Kenya
Ingle 2018	401174_aEPEC	ERR137823	Stool	Kenya
Ingle 2018	401250_aEPEC	ERR137790	Stool	Kenya
Ingle 2018	401352	ERR178199	Stool	Kenya
Ingle 2018	401363	ERR178179	Stool	Kenya
Ingle 2018	401480_aEPEC	ERR124657	Stool	Kenya
Ingle 2018	401553	ERR178155	Stool	Kenya
Ingle 2018	401596_aEPEC	ERR137791	Stool	Kenya
Ingle 2018	401686	ERR178200	Stool	Kenya
Ingle 2018	401709_aEPEC	ERR137824	Stool	Kenya
Ingle 2018	401886_aEPEC	ERR137792	Stool	Kenya
Ingle 2018	401907	ERR178201	Stool	Kenya
Ingle 2018	401938_aEPEC	ERR137793	Stool	Kenya
Ingle 2018	402048_aEPEC	ERR134532	Stool	Kenya
Ingle 2018	402058_aEPEC	ERR137825	Stool	Kenya
Ingle 2018	402074_aEPEC	ERR137794	Stool	Kenya
Ingle 2018	402078	ERR178180	Stool	Kenya
Ingle 2018	402097_aEPEC	ERR137826	Stool	Kenya
Ingle 2018	402099_aEPEC	ERR134533	Stool	Kenya
Ingle 2018	402138_aEPEC	ERR124662	Stool	Kenya
Ingle 2018	402227_aEPEC	ERR137827	Stool	Kenya
Ingle 2018	402248_aEPEC	ERR134534	Stool	Kenya
Ingle 2018	402403	ERR178181	Stool	Kenya
Ingle 2018	402480_aEPEC	ERR137795	Stool	Kenya
Ingle 2018	402605	ERR178194	Stool	Kenya
Ingle 2018	402617	ERR178156	Stool	Kenya
Ingle 2018	402635	ERR178157	Stool	Kenya

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Ingle 2018	402654_aEPEC	ERR134535	Stool	Kenya
Ingle 2018	402696	ERR178202	Stool	Kenya
Ingle 2018	402743_aEPEC	ERR137796	Stool	Kenya
Ingle 2018	402767	ERR178203	Stool	Kenya
Ingle 2018	402770_aEPEC	ERR134536	Stool	Kenya
Ingle 2018	402780_aEPEC	ERR137797	Stool	Kenya
Ingle 2018	402794	ERR178204	Stool	Kenya
Ingle 2018	402798	ERR178182	Stool	Kenya
Ingle 2018	402837	ERR178183	Stool	Kenya
Ingle 2018	402842	ERR178205	Stool	Kenya
Ingle 2018	402898	ERR178184	Stool	Kenya
Ingle 2018	402924	ERR178158	Stool	Kenya
Ingle 2018	402977_aEPEC	ERR137798	Stool	Kenya
Ingle 2018	403066	ERR178159	Stool	Kenya
Ingle 2018	403096_aEPEC	ERR137799	Stool	Kenya
Ingle 2018	403128_aEPEC	ERR134537	Stool	Kenya
Ingle 2018	403308_aEPEC	ERR134538	Stool	Kenya
Ingle 2018	403523	ERR178206	Stool	Kenya
Ingle 2018	403726_aEPEC	ERR137800	Stool	Kenya
Ingle 2018	403728	ERR178161	Stool	Kenya
Ingle 2018	500094	ERR178207	Stool	India
Ingle 2018	500095	ERR178208	Stool	India
Ingle 2018	500193	ERR178213	Stool	India
Ingle 2018	500197_aEPEC	ERR137828	Stool	India
Ingle 2018	500275_aEPEC	ERR134539	Stool	India
Ingle 2018	500618_aEPEC	ERR134540	Stool	India
Ingle 2018	500858_aEPEC	ERR134541	Stool	India
Ingle 2018	500864_aEPEC	ERR134542	Stool	India
Ingle 2018	500989	ERR178164	Stool	India
Ingle 2018	501016	ERR178195	Stool	India
Ingle 2018	501029_aEPEC	ERR134543	Stool	India
Ingle 2018	503023	ERR178196	Stool	India

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Ingle 2018	503028_aEPEC	ERR134544	Stool	India
Ingle 2018	503130_aEPEC	ERR137829	Stool	India
Ingle 2018	503225_aEPEC	ERR134545	Stool	India
Ingle 2018	503238_aEPEC	ERR137801	Stool	India
Ingle 2018	503256	ERR178197	Stool	India
Ingle 2018	503311_aEPEC	ERR124653	Stool	India
Ingle 2018	503320	ERR178219	Stool	India
Ingle 2018	503331	ERR178165	Stool	India
Ingle 2018	503459_aEPEC	ERR134546	Stool	India
Ingle 2018	503537_aEPEC	ERR124663	Stool	India
Ingle 2018	503662_aEPEC	ERR134547	Stool	India
Ingle 2018	503891_aEPEC	ERR137802	Stool	India
Ingle 2018	503947_aEPEC	ERR124654	Stool	India
Ingle 2018	504005_aEPEC	ERR137803	Stool	India
Ingle 2018	504180	ERR178166	Stool	India
Ingle 2018	504225_aEPEC	ERR134548	Stool	India
Ingle 2018	504300_aEPEC	ERR134549	Stool	India
Ingle 2018	504324	ERR178167	Stool	India
Ingle 2018	504449_aEPEC	ERR134550	Stool	India
Ingle 2018	504528	ERR178168	Stool	India
Ingle 2018	504647_aEPEC	ERR134551	Stool	India
Ingle 2018	504718	ERR178169	Stool	India
Ingle 2018	504821_aEPEC	ERR134552	Stool	India
Ingle 2018	504888_aEPEC	ERR134553	Stool	India
Ingle 2018	504925_aEPEC	ERR124664	Stool	India
Ingle 2018	505148	ERR178170	Stool	India
Ingle 2018	505393_aEPEC	ERR124655	Stool	India
Ingle 2018	505513_aEPEC	ERR124656	Stool	India
Ingle 2018	505545	ERR178171	Stool	India
Ingle 2018	602206	ERR178172	Stool	Bangladesh
Ingle 2018	602370_aEPEC	ERR134554	Stool	Bangladesh

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Ingle 2018	700149	ERR178214	Stool	Pakistan
Ingle 2018	700337_aEPEC	ERR134555	Stool	Pakistan
Ingle 2018	700495_aEPEC	ERR134556	Stool	Pakistan
Ingle 2018	700851	ERR178173	Stool	Pakistan
Ingle 2018	700863	ERR178215	Stool	Pakistan
Ingle 2018	702161_aEPEC	ERR134558	Stool	Pakistan
Ingle 2018	702328	ERR178174	Stool	Pakistan
Ingle 2018	702566	ERR178175	Stool	Pakistan
Ingle 2018	702745_aEPEC	ERR137804	Stool	Pakistan
Ingle 2018	702797	ERR178186	Stool	Pakistan
Ingle 2018	702890_aEPEC	ERR137805	Stool	Pakistan
Ingle 2018	702898_aEPEC	ERR137806	Stool	Pakistan
Ingle 2018	702971	ERR178185	Stool	Pakistan
Ingle 2018	703063	ERR178209	Stool	Pakistan
Ingle 2018	703108	ERR178187	Stool	Pakistan
Ingle 2018	703128	ERR178188	Stool	Pakistan
Ingle 2018	703258_aEPEC	ERR134559	Stool	Pakistan
Ingle 2018	703273	ERR178191	Stool	Pakistan
Ingle 2018	703753	ERR178189	Stool	Pakistan
Ingle 2018	703975_aEPEC	ERR134560	Stool	Pakistan
Ingle 2018	G100788-1A	ERR175731	Stool	Gambia
Ingle 2018	G302544	ERR178226	Stool	Mozambique
Ingle 2018	G302551	ERR178225	Stool	Mozambique
Ingle 2018	G303212	ERR175730	Stool	Mozambique
Ingle 2018	G400792	ERR175725	Stool	Kenya
Ingle 2018	G400871	ERR175724	Stool	Kenya
Ingle 2018	G401436	ERR175727	Stool	Kenya
Ingle 2018	G401529	ERR178227	Stool	Kenya
Ingle 2018	G500007	ERR178223	Stool	India
Ingle 2018	G500297-1	ERR175733	Stool	India
Ingle 2018	G500407	ERR178221	Stool	India
Ingle 2018	G500830	ERR175728	Stool	India

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Ingle 2018	G503854	ERR178224	Stool	India
Ingle 2018	G504540	ERR178222	Stool	India
Ingle 2018	G603423	ERR178228	Stool	Bangladesh
Ingle 2018	G702074-1	ERR175734	Stool	Pakistan
Ingle 2018	G702074-2	ERR175735	Stool	Pakistan
Ingle 2018	R203092-3A	ERR175736	Stool	Mali
Ingle 2018	R203092-3B	ERR175737	Stool	Mali
Ingle 2018	R302583-2A	ERR175738	Stool	Mozambique
Ingle 2018	R302583-2B	ERR175739	Stool	Mozambique
Ingle 2018	R402077	ERR175726	Stool	Kenya
Ingle 2018	R503696	ERR175729	Stool	India
Mentzer 2014	E_1003	ERR054711	Stool	Egypt
Mentzer 2014	E_1009	ERR054712	Stool	Egypt
Mentzer 2014	E_1018	ERR084463	Stool	Egypt
Mentzer 2014	E_1034	ERR052911	Stool	Egypt
Mentzer 2014	E_1057CFn	ERR119471	Stool	Egypt
Mentzer 2014	E_106	ERR054666	Stool	unknown
Mentzer 2014	E_1072CFn	ERR119472	Stool	Egypt
Mentzer 2014	E_1074	ERR052912	Stool	Egypt
Mentzer 2014	E_1085	ERR052913	Stool	Egypt
Mentzer 2014	E_1091	ERR052914	Stool	Egypt
Mentzer 2014	E_110	ERR054678	Stool	Bangladesh
Mentzer 2014	E_1101CFn	ERR119473	Stool	Egypt
Mentzer 2014	E_1102CFn	ERR119474	Stool	Egypt
Mentzer 2014	E_1111	ERR052915	Stool	Egypt
Mentzer 2014	E_1167CFn	ERR119475	Stool	Egypt
Mentzer 2014	E_1169CFn	ERR119476	Stool	Egypt
Mentzer 2014	E_1189	ERR052916	Stool	Egypt
Mentzer 2014	E_1193CFn	ERR119477	Stool	Egypt
Mentzer 2014	E_1242CFn	ERR119478	Stool	Egypt
Mentzer 2014	E_1245	ERR052917	Stool	Egypt
Mentzer 2014	E_1248CFn	ERR119479	Stool	Egypt

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Mentzer 2014	E_1258CFn	ERR119480	Stool	Egypt
Mentzer 2014	E_126	ERR054679	Stool	unknown
Mentzer 2014	E_1264CFn	ERR119481	Stool	Egypt
Mentzer 2014	E_1281CFn	ERR119482	Stool	Egypt
Mentzer 2014	E_1282CFn	ERR119483	Stool	Egypt
Mentzer 2014	E_1285CFn	ERR119484	Stool	Egypt
Mentzer 2014	E_1287	ERR052918	Stool	Egypt
Mentzer 2014	E_129	ERR054680	Stool	Zaire
Mentzer 2014	E_1298	ERR052919	Stool	Egypt
Mentzer 2014	E_1316	ERR161000	Stool	Nepal
Mentzer 2014	E_133	ERR054681	Stool	unknown
Mentzer 2014	E_1334	ERR084464	Stool	China
Mentzer 2014	E_135	ERR054682	Stool	unknown
Mentzer 2014	E_1352CFn	ERR119485	Stool	Egypt
Mentzer 2014	E_1355CFn	ERR119486	Stool	Egypt
Mentzer 2014	E_1356CFn	ERR119487	Stool	Egypt
Mentzer 2014	E_1360_sec	ERR178234	Stool	Tunisia
Mentzer 2014	E_1362CFn	ERR119489	Stool	Egypt
Mentzer 2014	E_1363	ERR084466	Stool	Egypt
Mentzer 2014	E_1365CFn	ERR119490	Stool	Egypt
Mentzer 2014	E_1373	ERR052920	Stool	Indonesia
Mentzer 2014	E_1392	ERR052921	Stool	Indonesia
Mentzer 2014	E_1398CFn	ERR119491	Stool	Indonesia
Mentzer 2014	E_1407CFn	ERR119492	Stool	Mexico
Mentzer 2014	E_141	ERR054683	Stool	Burma
Mentzer 2014	E_1429tiny	ERR217371	Stool	Venezuela
Mentzer 2014	E_143	ERR054684	Stool	Japan
Mentzer 2014	E_1432G	ERR164830	Stool	Venezuela
Mentzer 2014	E_1432w	ERR164829	Stool	Venezuela
Mentzer 2014	E_1433	ERR084468	Stool	Morocco
Mentzer 2014	E_1460	ERR084469	Stool	Indonesia

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Mentzer 2014	E_151	ERR054685	Stool	Japan
Mentzer 2014	E_1524	ERR084470	Stool	Argentina
Mentzer 2014	E_1525CFn	ERR119496	Stool	Argentina
Mentzer 2014	E_1526CFn	ERR119497	Stool	Argentina
Mentzer 2014	E_1527	ERR084471	Stool	Argentina
Mentzer 2014	E_1532CFn	ERR119498	Stool	Argentina
Mentzer 2014	E_1533CFn	ERR119499	Stool	Argentina
Mentzer 2014	E_1534CFn	ERR119500	Stool	Argentina
Mentzer 2014	E_1535	ERR084472	Stool	Argentina
Mentzer 2014	E_1541	ERR052922	Stool	Argentina
Mentzer 2014	E_1542CFn	ERR119501	Stool	Argentina
Mentzer 2014	E_1543CFn	ERR119502	Stool	Argentina
Mentzer 2014	E_1544CFn	ERR119503	Stool	Argentina
Mentzer 2014	E_1548	ERR052923	Stool	Argentina
Mentzer 2014	E_1556CFn	ERR119504	Stool	Argentina
Mentzer 2014	E_1561CFn	ERR119505	Stool	Argentina
Mentzer 2014	E_1564CFn	ERR119506	Stool	Argentina
Mentzer 2014	E_1573CFn	ERR119508	Stool	Argentina
Mentzer 2014	E_1574CFn	ERR119509	Stool	Argentina
Mentzer 2014	E_1576CFn	ERR119510	Stool	Argentina
Mentzer 2014	E_157CFn	ERR119507	Stool	Japan
Mentzer 2014	E_1580CFn	ERR119511	Stool	Argentina
Mentzer 2014	E_1581CFn	ERR119512	Stool	Argentina
Mentzer 2014	E_1582CFn	ERR119513	Stool	Argentina
Mentzer 2014	E_1585CFn	ERR119514	Stool	Argentina
Mentzer 2014	E_1586CFn	ERR119515	Stool	Argentina
Mentzer 2014	E_1587CFn	ERR119516	Stool	Argentina
Mentzer 2014	E_1592CFn	ERR119518	Stool	Argentina
Mentzer 2014	E_1593	ERR084473	Stool	Argentina
Mentzer 2014	E_1594CFn	ERR119519	Stool	Argentina
Mentzer 2014	E_1596CFn	ERR119520	Stool	Argentina
Mentzer 2014	E_1597CFn	ERR119521	Stool	Argentina

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Mentzer 2014	E_1599CFn	ERR119522	Stool	Argentina
Mentzer 2014	E_159CFn	ERR119517	Stool	Japan
Mentzer 2014	E_1600CFn	ERR119524	Stool	Argentina
Mentzer 2014	E_1604CFn	ERR119525	Stool	Argentina
Mentzer 2014	E_1607CFn	ERR119526	Stool	Argentina
Mentzer 2014	E_1609CFn	ERR119527	Stool	Argentina
Mentzer 2014	E_1611CFn	ERR119528	Stool	Argentina
Mentzer 2014	E_1615CFn	ERR119529	Stool	Argentina
Mentzer 2014	E_1616CFn	ERR119530	Stool	Argentina
Mentzer 2014	E_1617CFn	ERR119531	Stool	Argentina
Mentzer 2014	E_1620	ERR084474	Stool	Argentina
Mentzer 2014	E_1623CFn	ERR119532	Stool	Indonesia
Mentzer 2014	E_1624	ERR052924	Stool	Indonesia
Mentzer 2014	E_1625CFn	ERR119533	Stool	Indonesia
Mentzer 2014	E_1628CFn	ERR119534	Stool	Indonesia
Mentzer 2014	E_1634CFn	ERR119535	Stool	Indonesia
Mentzer 2014	E_1635	ERR084475	Stool	Indonesia
Mentzer 2014	E_1637CFn	ERR119536	Stool	Indonesia
Mentzer 2014	E_1638CFn	ERR119537	Stool	Indonesia
Mentzer 2014	E_1640CFn	ERR119538	Stool	Indonesia
Mentzer 2014	E_1641CFn	ERR119539	Stool	Indonesia
Mentzer 2014	E_1642CFn	ERR119540	Stool	Indonesia
Mentzer 2014	E_1646	ERR052925	Stool	Indonesia
Mentzer 2014	E_1647CFn	ERR119541	Stool	Indonesia
Mentzer 2014	E_1648CFn	ERR119542	Stool	Indonesia
Mentzer 2014	E_1649	ERR084476	Stool	Indonesia
Mentzer 2014	E_1650CFn	ERR119543	Stool	Indonesia
Mentzer 2014	E_1654	ERR054665	Stool	Indonesia
Mentzer 2014	E_1657	ERR084477	Stool	Indonesia
Mentzer 2014	E_1659CFn	ERR119544	Stool	Indonesia
Mentzer 2014	E_1661CFn	ERR119545	Stool	Indonesia
Mentzer 2014	E_1666CFn	ERR119546	Stool	Indonesia

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Mentzer 2014	E_1667	ERR084478	Stool	Indonesia
Mentzer 2014	E_1673CFn	ERR119548	Stool	Indonesia
Mentzer 2014	E_1674CFn	ERR119549	Stool	Indonesia
Mentzer 2014	E_1679sec	ERR217373	Stool	Indonesia
Mentzer 2014	E_167CFn	ERR119547	Stool	Japan
Mentzer 2014	E_1682CFn	ERR119551	Stool	Indonesia
Mentzer 2014	E_1684CFn	ERR119552	Stool	Indonesia
Mentzer 2014	E_1690CFn	ERR119553	Stool	Indonesia
Mentzer 2014	E_1712CFn	ERR119554	Stool	Bangladesh
Mentzer 2014	E_1716	ERR052926	Stool	Bangladesh
Mentzer 2014	E_1724	ERR084479	Stool	Bangladesh
Mentzer 2014	E_1735CFn	ERR119555	Stool	Bangladesh
Mentzer 2014	E_1736CFn	ERR119556	Stool	Bangladesh
Mentzer 2014	E_1739CFn	ERR119557	Stool	Bangladesh
Mentzer 2014	E_1741	ERR084480	Stool	Bangladesh
Mentzer 2014	E_1744CFn	ERR119558	Stool	Bangladesh
Mentzer 2014	E_1750	ERR084481	Stool	Bangladesh
Mentzer 2014	E_1752CFn	ERR119559	Stool	Bangladesh
Mentzer 2014	E_1760	ERR084482	Stool	Bangladesh
Mentzer 2014	E_1779	ERR052927	Stool	Bangladesh
Mentzer 2014	E_1784	ERR052928	Stool	Bangladesh
Mentzer 2014	E_1795	ERR084483	Stool	Bangladesh
Mentzer 2014	E_1797	ERR084484	Stool	Bangladesh
Mentzer 2014	E_1841	ERR084485	Stool	Bangladesh
Mentzer 2014	E_1871CFn	ERR119560	Stool	Bangladesh
Mentzer 2014	E_1883	ERR084486	Stool	Bangladesh
Mentzer 2014	E_1918	ERR084487	Stool	Bangladesh
Mentzer 2014	E_1939	ERR084488	Stool	Bangladesh
Mentzer 2014	E_1947	ERR084489	Stool	Bangladesh
Mentzer 2014	E_1961CFn	ERR119561	Stool	Bangladesh
Mentzer 2014	E_1994	ERR084490	Stool	Bangladesh

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Mentzer 2014	E_2088	ERR084491	Stool	Bangladesh
Mentzer 2014	E_2092	ERR089723	Stool	Bangladesh
Mentzer 2014	E_21	ERR054667	Stool	Bangladesh
Mentzer 2014	E_2108CFn	ERR119562	Stool	Bangladesh
Mentzer 2014	E_2110CFn	ERR119563	Stool	Bangladesh
Mentzer 2014	E_2118	ERR089724	Stool	Bangladesh
Mentzer 2014	E_2121CFn	ERR119564	Stool	Bangladesh
Mentzer 2014	E_2131	ERR089725	Stool	Bangladesh
Mentzer 2014	E_2185	ERR089726	Stool	Bolivia
Mentzer 2014	E_220	ERR054686	Stool	Japan
Mentzer 2014	E_2219	ERR089727	Stool	Bolivia
Mentzer 2014	E_222CFn	ERR119380	Stool	Japan
Mentzer 2014	E_223CFn	ERR119381	Stool	Japan
Mentzer 2014	E_224	ERR054687	Stool	Japan
Mentzer 2014	E_2256	ERR089728	Stool	Thailand
Mentzer 2014	E_2339	ERR089729	Stool	Bolivia
Mentzer 2014	E_2347	ERR089730	Stool	Bolivia
Mentzer 2014	E_2348	ERR089731	Stool	Bolivia
Mentzer 2014	E_2362_sec	ERR178236	Stool	Bolivia
Mentzer 2014	E_237	ERR054688	Stool	Japan
Mentzer 2014	E_2370sec	ERR217375	Stool	Japan
Mentzer 2014	E_2386	ERR089732	Stool	Bolivia
Mentzer 2014	E_2397	ERR089733	Stool	Bolivia
Mentzer 2014	E_239CFn	ERR119382	Stool	Japan
Mentzer 2014	E_2439	ERR164832	Stool	Bolivia
Mentzer 2014	E_251	ERR054689	Stool	Japan
Mentzer 2014	E_263CFn	ERR119383	Stool	Japan
Mentzer 2014	E_272	ERR054690	Stool	Japan
Mentzer 2014	E_28	ERR054668	Stool	Bangladesh
Mentzer 2014	E_2980	ERR089734	Stool	Bangladesh
Mentzer 2014	E_2981_sec	ERR178239	Stool	Bangladesh
Mentzer 2014	E_329CFn	ERR119384	Stool	Mexico

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Mentzer 2014	E_330CFn	ERR119385	Stool	Mexico
Mentzer 2014	E_333	ERR049162	Stool	Mexico
Mentzer 2014	E_335CFn	ERR119386	Stool	Mexico
Mentzer 2014	E_336CFn	ERR119387	Stool	Mexico
Mentzer 2014	E_340CFn	ERR119388	Stool	Mexico
Mentzer 2014	E_343CFn	ERR119389	Stool	Mexico
Mentzer 2014	E_344	ERR049163	Stool	Mexico
Mentzer 2014	E_351CFn	ERR119390	Stool	Mexico
Mentzer 2014	E_354CFn	ERR119391	Stool	Mexico
Mentzer 2014	E_356CFn	ERR119392	Stool	Mexico
Mentzer 2014	E_36	ERR054669	Stool	Bangladesh
Mentzer 2014	E_360CFn	ERR119393	Stool	Mexico
Mentzer 2014	E_361CFn	ERR119394	Stool	Mexico
Mentzer 2014	E_370	ERR049164	Stool	Guatemala
Mentzer 2014	E_390CFn	ERR119395	Stool	Guatemala
Mentzer 2014	E_391CFn	ERR119396	Stool	Guatemala
Mentzer 2014	E_399CFn	ERR119397	Stool	Guatemala
Mentzer 2014	E_405CFn	ERR119398	Stool	Guatemala
Mentzer 2014	E_415CFn	ERR119399	Stool	Guatemala
Mentzer 2014	E_416	ERR049165	Stool	Guatemala
Mentzer 2014	E_425CFn	ERR119400	Stool	Guatemala
Mentzer 2014	E_445CFn	ERR119401	Stool	Guatemala
Mentzer 2014	E_45	ERR054670	Stool	Bangladesh
Mentzer 2014	E_451CFn	ERR119402	Stool	Guatemala
Mentzer 2014	E_471	ERR049166	Stool	Guatemala
Mentzer 2014	E_5089	ERR164833	Stool	Bangladesh
Mentzer 2014	E_509	ERR178229	Stool	Mexico
Mentzer 2014	E_513CFn	ERR119404	Stool	Mexico
Mentzer 2014	E_517	ERR049167	Stool	Mexico
Mentzer 2014	E_519CFn	ERR119405	Stool	Mexico
Mentzer 2014	E_520CFn	ERR119406	Stool	Mexico
Mentzer 2014	E_523CFn	ERR119407	Stool	Mexico

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Mentzer 2014	E_527CFn	ERR119408	Stool	Mexico
Mentzer 2014	E_528CFn	ERR119409	Stool	Mexico
Mentzer 2014	E_529CFn	ERR119410	Stool	Mexico
Mentzer 2014	E_54	ERR049158	Stool	Bangladesh
Mentzer 2014	E_554	ERR049168	Stool	Mexico
Mentzer 2014	E_562	ERR049169	Stool	Mexico
Mentzer 2014	E_563	ERR049170	Stool	Mexico
Mentzer 2014	E_604CFn	ERR119411	Stool	Guatemala
Mentzer 2014	E_616CFn	ERR119412	Stool	Guatemala
Mentzer 2014	E_618CFn	ERR119413	Stool	Guatemala
Mentzer 2014	E_620CFn	ERR119414	Stool	Guatemala
Mentzer 2014	E_621	ERR178230	Stool	Guatemala
Mentzer 2014	E_622CFn	ERR119416	Stool	Guatemala
Mentzer 2014	E_626CFn	ERR119417	Stool	Guatemala
Mentzer 2014	E_628CFn	ERR119418	Stool	Guatemala
Mentzer 2014	E_632	ERR049171	Stool	Guatemala
Mentzer 2014	E_636	ERR049172	Stool	Guatemala
Mentzer 2014	E_645CFn	ERR119419	Stool	Guatemala
Mentzer 2014	E_655	ERR049173	Stool	Guatemala
Mentzer 2014	E_658CFn	ERR119420	Stool	Guatemala
Mentzer 2014	E_659CFn	ERR119421	Stool	Guatemala
Mentzer 2014	E_66	ERR054671	Stool	Bangladesh
Mentzer 2014	E_662CFn	ERR119422	Stool	Guatemala
Mentzer 2014	E_70	ERR049159	Stool	Bangladesh
Mentzer 2014	E_703CFn	ERR119423	Stool	Guatemala
Mentzer 2014	E_704CFn	ERR119424	Stool	Guatemala
Mentzer 2014	E_705CFn	ERR119425	Stool	Guatemala
Mentzer 2014	E_71	ERR049160	Stool	Bangladesh
Mentzer 2014	E_710	ERR178231	Stool	Guatemala
Mentzer 2014	E_74	ERR054672	Stool	Bangladesh
Mentzer 2014	E_79	ERR054673	Stool	Bangladesh

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Mentzer 2014	E_8	ERR049156	Stool	Bangladesh
Mentzer 2014	E_806	ERR054691	Stool	Guatemala
Mentzer 2014	E_810	ERR054692	Stool	Guatemala
Mentzer 2014	E_811	ERR178232	Stool	Guatemala
Mentzer 2014	E_812	ERR054693	Stool	Guatemala
Mentzer 2014	E_816	ERR054694	Stool	Guatemala
Mentzer 2014	E_818CFn	ERR119428	Stool	Guatemala
Mentzer 2014	E_819CFn	ERR119429	Stool	Guatemala
Mentzer 2014	E_821CFn	ERR119430	Stool	Guatemala
Mentzer 2014	E_822	ERR054695	Stool	Guatemala
Mentzer 2014	E_828CFn	ERR119431	Stool	Guatemala
Mentzer 2014	E_833CFn	ERR119432	Stool	Guatemala
Mentzer 2014	E_841CFn	ERR119433	Stool	Guatemala
Mentzer 2014	E_842	ERR054696	Stool	Guatemala
Mentzer 2014	E_85	ERR054674	Stool	Bangladesh
Mentzer 2014	E_855CFn	ERR119434	Stool	Guatemala
Mentzer 2014	E_856	ERR054697	Stool	Guatemala
Mentzer 2014	E_858	ERR054698	Stool	Guatemala
Mentzer 2014	E_860CFn	ERR119435	Stool	Guatemala
Mentzer 2014	E_863	ERR049174	Stool	Guatemala
Mentzer 2014	E_865CFn	ERR119436	Stool	Guatemala
Mentzer 2014	E_867	ERR054699	Stool	Guatemala
Mentzer 2014	E_87	ERR054675	Stool	Bangladesh
Mentzer 2014	E_871	ERR054700	Stool	Guatemala
Mentzer 2014	E_873CFn	ERR119437	Stool	Guatemala
Mentzer 2014	E_876CFn	ERR119438	Stool	Guatemala
Mentzer 2014	E_877	ERR049175	Stool	Guatemala
Mentzer 2014	E_879	ERR049176	Stool	Guatemala
Mentzer 2014	E_88	ERR049161	Stool	Bangladesh
Mentzer 2014	E_881CFn	ERR119439	Stool	Guatemala
Mentzer 2014	E_882	ERR054701	Stool	Guatemala
Mentzer 2014	E_883	ERR054702	Stool	Guatemala

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Mentzer 2014	E_884CFn	ERR119441	Stool	Guatemala
Mentzer 2014	E_885CFn	ERR119442	Stool	Guatemala
Mentzer 2014	E_887CFn	ERR119443	Stool	Guatemala
Mentzer 2014	E_888CFn	ERR119444	Stool	Guatemala
Mentzer 2014	E_890CFn	ERR119445	Stool	Guatemala
Mentzer 2014	E_891CFn	ERR119446	Stool	Guatemala
Mentzer 2014	E_892CFn	ERR119447	Stool	Guatemala
Mentzer 2014	E_893CFn	ERR119448	Stool	Guatemala
Mentzer 2014	E_895	ERR054703	Stool	Guatemala
Mentzer 2014	E_897	ERR049177	Stool	Guatemala
Mentzer 2014	E_898CFn	ERR119449	Stool	Guatemala
Mentzer 2014	E_899CFn	ERR119450	Stool	Guatemala
Mentzer 2014	E_900	ERR054704	Stool	Guatemala
Mentzer 2014	E_901	ERR054705	Stool	Guatemala
Mentzer 2014	E_903	ERR054706	Stool	Guatemala
Mentzer 2014	E_907	ERR054707	Stool	Guatemala
Mentzer 2014	E_908CFn	ERR119452	Stool	Guatemala
Mentzer 2014	E_916	ERR049178	Stool	Guatemala
Mentzer 2014	E_917	ERR049179	Stool	Guatemala
Mentzer 2014	E_920	ERR054708	Stool	Guatemala
Mentzer 2014	E_924CFn	ERR119453	Stool	Guatemala
Mentzer 2014	E_925	ERR052905	Stool	Guatemala
Mentzer 2014	E_927	ERR052906	Stool	Egypt
Mentzer 2014	E_928	ERR054709	Stool	Egypt
Mentzer 2014	E_934CFn	ERR119455	Stool	Egypt
Mentzer 2014	E_935CFn	ERR119456	Stool	Egypt
Mentzer 2014	E_936CFn	ERR119457	Stool	Egypt
Mentzer 2014	E_938	ERR052907	Stool	Egypt
Mentzer 2014	E_939CFn	ERR119458	Stool	Egypt
Mentzer 2014	E_940	ERR052908	Stool	Egypt
Mentzer 2014	E_941CFn	ERR119459	Stool	Egypt
Mentzer 2014	E_943	ERR054710	Stool	Egypt

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Mentzer 2014	E_944CFn	ERR119460	Stool	Egypt
Mentzer 2014	E_945CFn	ERR119461	Stool	Egypt
Mentzer 2014	E_947CFn	ERR119462	Stool	Egypt
Mentzer 2014	E_949CFn	ERR119463	Stool	Egypt
Mentzer 2014	E_952CFn	ERR119464	Stool	Egypt
Mentzer 2014	E_953	ERR052909	Stool	Egypt
Mentzer 2014	E_955CFn	ERR119465	Stool	Egypt
Mentzer 2014	E_956CFn	ERR119466	Stool	Egypt
Mentzer 2014	E_957	ERR164828	Stool	Egypt
Mentzer 2014	E_97	ERR054676	Stool	Bangladesh
Mentzer 2014	E_978CFn	ERR119468	Stool	Egypt
Mentzer 2014	E_986	ERR052910	Stool	Egypt
Mentzer 2014	E_99	ERR054677	Stool	Bangladesh
Mentzer 2014	E_995	ERR160999	Stool	Egypt
Mentzer 2014	E_996CFn	ERR119469	Stool	Egypt
Mentzer 2014	E_998CFn	ERR119470	Stool	Egypt
Mentzer 2014	E160CFn	ERR119523	Stool	Japan
Mentzer 2014	E2367CFn	ERR119566	Stool	Bolivia
Mentzer 2014	E2371CFn	ERR119568	Stool	Bolivia
Mentzer 2014	E2377CFn	ERR119569	Stool	Bolivia
Mentzer 2014	E2388CFn	ERR119570	Stool	Bolivia
Mentzer 2014	E2392CFn	ERR119571	Stool	Bolivia
Mentzer 2014	E2393CFn	ERR119572	Stool	Bolivia
Mentzer 2014	E2395CFn	ERR119573	Stool	Bolivia
Mentzer 2014	E2404CFn	ERR119574	Stool	Bolivia
Mentzer 2014	E2405CFn	ERR119575	Stool	Bolivia
Mentzer 2014	E3015CFn	ERR119577	Stool	Egypt
Mentzer 2014	E4134CFn	ERR119578	Stool	Israel
Mentzer 2014	E5049	ERR089738	Stool	India
Mentzer 2014	E5051	ERR089739	Stool	India
Mentzer 2014	E5052	ERR089740	Stool	India

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Mentzer 2014	E5080	ERR089741	Stool	Bangladesh
Mentzer 2014	E5081	ERR089742	Stool	Bangladesh
Mentzer 2014	E5082	ERR089743	Stool	Bangladesh
Mentzer 2014	E5084	ERR089744	Stool	Bangladesh
Mentzer 2014	E5085	ERR089745	Stool	Bangladesh
Mentzer 2014	E5086	ERR089746	Stool	Bangladesh
Mentzer 2014	E5087	ERR089747	Stool	Bangladesh
Mentzer 2014	E5088	ERR089748	Stool	Bangladesh
Mentzer 2014	ILBEcoli5442571	ERR279354	Stool	Kenya
Mentzer 2014	ILBEcoli5442572	ERR279355	Stool	Kenya
Mentzer 2014	ILBEcoli5442573	ERR279356	Stool	Kenya
Mentzer 2014	ILBEcoli5442574	ERR279357	Stool	Kenya
Mentzer 2014	ILBEcoli5442575	ERR279358	Stool	Kenya
Mentzer 2014	ILBEcoli5442576	ERR279359	Stool	Kenya
Mentzer 2014	ILBEcoli5442577	ERR279360	Stool	Kenya
Mentzer 2014	ILBEcoli5442578	ERR279361	Stool	Kenya
Mentzer 2014	ILBEcoli5442579	ERR279362	Stool	Kenya
Mentzer 2014	ILBEcoli5442580	ERR279363	Stool	Kenya
Mentzer 2014	ILBEcoli5442581	ERR279364	Stool	Kenya
Mentzer 2014	ILBEcoli5442582	ERR279365	Stool	Kenya
Mentzer 2014	ILBEcoli5442583	ERR279366	Stool	Kenya
Mentzer 2014	ILBEcoli5442587	ERR279370	Stool	Guinea Bissau
Mentzer 2014	ILBEcoli5442588	ERR279371	Stool	Guinea Bissau
Mentzer 2014	ILBEcoli5442589	ERR279372	Stool	Guinea Bissau
Mentzer 2014	ILBEcoli5442590	ERR279373	Stool	Guinea Bissau
Musicha 2017	3487STDY6036382	ERR926351	Blood	Malawi
Musicha 2017	3487STDY6036383	ERR926352	Blood	Malawi
Musicha 2017	3487STDY6036384	ERR926353	Blood	Malawi
Musicha 2017	3487STDY6036385	ERR926354	CSF	Malawi
Musicha 2017	3487STDY6036386	ERR926355	Blood	Malawi
Musicha 2017	3487STDY6036387	ERR926356	Blood	Malawi
Musicha 2017	3487STDY6036388	ERR926357	Blood	Malawi

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Musicha 2017	3487STDY6036389	ERR926358	Blood	Malawi
Musicha 2017	3487STDY6036390	ERR926359	CSF	Malawi
Musicha 2017	3487STDY6036391	ERR926360	Blood	Malawi
Musicha 2017	3487STDY6036392	ERR926361	Blood	Malawi
Musicha 2017	3487STDY6036393	ERR926362	Blood	Malawi
Musicha 2017	3487STDY6036394	ERR926363	Blood	Malawi
Musicha 2017	3487STDY6036395	ERR926364	Blood	Malawi
Musicha 2017	3487STDY6036396	ERR926365	CSF	Malawi
Musicha 2017	3487STDY6036397	ERR926366	Blood	Malawi
Musicha 2017	3487STDY6036398	ERR926367	Blood	Malawi
Musicha 2017	3487STDY6036399	ERR926368	Blood	Malawi
Musicha 2017	3487STDY6036400	ERR926369	CSF	Malawi
Musicha 2017	3487STDY6036403	ERR926372	CSF	Malawi
Musicha 2017	3487STDY6036404	ERR926373	Blood	Malawi
Musicha 2017	3487STDY6036405	ERR926374	CSF	Malawi
Musicha 2017	3487STDY6036406	ERR926375	Blood	Malawi
Musicha 2017	3487STDY6036407	ERR926376	CSF	Malawi
Musicha 2017	3487STDY6036408	ERR926377	Blood	Malawi
Musicha 2017	3487STDY6036409	ERR926378	Blood	Malawi
Musicha 2017	3487STDY6036410	ERR926379	Blood	Malawi
Musicha 2017	3487STDY6036411	ERR926380	Blood	Malawi
Musicha 2017	3487STDY6036412	ERR926381	Blood	Malawi
Musicha 2017	3487STDY6036413	ERR971988	CSF	Malawi
Musicha 2017	3487STDY6036414	ERR926382	Blood	Malawi
Musicha 2017	3487STDY6036415	ERR926383	CSF	Malawi
Musicha 2017	3487STDY6036416	ERR926384	CSF	Malawi
Musicha 2017	3487STDY6036417	ERR926385	CSF	Malawi
Musicha 2017	3487STDY6036418	ERR926386	CSF	Malawi
Musicha 2017	3487STDY6036420	ERR926388	CSF	Malawi
Musicha 2017	3487STDY6036421	ERR926389	Blood	Malawi
Musicha 2017	3487STDY6036422	ERR926390	CSF	Malawi
Musicha 2017	3487STDY6036423	ERR926391	CSF	Malawi

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Musicha 2017	3487STDY6036424	ERR926392	Blood	Malawi
Musicha 2017	3487STDY6036425	ERR926393	Blood	Malawi
Musicha 2017	3487STDY6036426	ERR926394	Blood	Malawi
Musicha 2017	3487STDY6036427	ERR926395	Blood	Malawi
Musicha 2017	3487STDY6036428	ERR926396	Blood	Malawi
Musicha 2017	3487STDY6036429	ERR926397	Blood	Malawi
Musicha 2017	3487STDY6036430	ERR926398	Blood	Malawi
Musicha 2017	3487STDY6036431	ERR926399	Blood	Malawi
Musicha 2017	3487STDY6036432	ERR926400	Blood	Malawi
Musicha 2017	3487STDY6036433	ERR926401	CSF	Malawi
Musicha 2017	3487STDY6036434	ERR926402	CSF	Malawi
Musicha 2017	3487STDY6036435	ERR926403	CSF	Malawi
Musicha 2017	3487STDY6036436	ERR926404	Blood	Malawi
Musicha 2017	3487STDY6036437	ERR926405	Blood	Malawi
Musicha 2017	3487STDY6036438	ERR926406	Blood	Malawi
Musicha 2017	3487STDY6036440	ERR926408	CSF	Malawi
Musicha 2017	3487STDY6036441	ERR926409	Blood	Malawi
Musicha 2017	3487STDY6036443	ERR926411	Blood	Malawi
Musicha 2017	3487STDY6036444	ERR926412	Blood	Malawi
Musicha 2017	3487STDY6036445	ERR926413	RS	Malawi
Musicha 2017	3487STDY6036446	ERR971989	Blood	Malawi
Musicha 2017	3487STDY6036447	ERR926414	CSF	Malawi
Musicha 2017	3487STDY6036448	ERR926415	Blood	Malawi
Musicha 2017	3487STDY6036449	ERR926416	Blood	Malawi
Musicha 2017	3487STDY6036450	ERR926417	RS	Malawi
Musicha 2017	3487STDY6036451	ERR926418	CSF	Malawi
Musicha 2017	3487STDY6036452	ERR926419	Blood	Malawi
Musicha 2017	3487STDY6036453	ERR926420	Blood	Malawi
Musicha 2017	3487STDY6036454	ERR971990	Blood	Malawi
Musicha 2017	3487STDY6036455	ERR926421	CSF	Malawi
Musicha 2017	3487STDY6036456	ERR971991	Blood	Malawi

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Musicha 2017	3487STDY6036457	ERR926422	RS	Malawi
Musicha 2017	3487STDY6036458	ERR926423	Blood	Malawi
Musicha 2017	3487STDY6036460	ERR926425	CSF	Malawi
Musicha 2017	3487STDY6036461	ERR926426	Blood	Malawi
Musicha 2017	3487STDY6036462	ERR971992	Blood	Malawi
Musicha 2017	3487STDY6036463	ERR926427	CSF	Malawi
Musicha 2017	3487STDY6036464	ERR926428	Blood	Malawi
Musicha 2017	3487STDY6036465	ERR926429	Blood	Malawi
Musicha 2017	3487STDY6036466	ERR926430	RS	Malawi
Musicha 2017	3487STDY6036467	ERR926431	RS	Malawi
Musicha 2017	3487STDY6036468	ERR926432	Blood	Malawi
Musicha 2017	3487STDY6036470	ERR926434	Blood	Malawi
Musicha 2017	3487STDY6036471	ERR926435	RS	Malawi
Musicha 2017	3487STDY6036473	ERR971994	RS	Malawi
Musicha 2017	3487STDY6036486	ERR926444	Blood	Malawi
Musicha 2017	3487STDY6036506	ERR971962	Blood	Malawi
Musicha 2017	3487STDY6036508	ERR971963	Blood	Malawi
Musicha 2017	3487STDY6036519	ERR972008	Blood	Malawi
Musicha 2017	3487STDY6036520	ERR971966	Blood	Malawi
Musicha 2017	3487STDY6036526	ERR971968	Blood	Malawi
Musicha 2017	3487STDY6036533	ERR971970	Blood	Malawi
Musicha 2017	3487STDY6036547	ERR971979	Blood	Malawi
Musicha 2017	3487STDY6036565	ERR971987	RS	Malawi
Runchaeron 2017	3898STDY6199571	ERR1218581	Urine	Thailand
Runchaeron 2017	3898STDY6199572	ERR1218582	Urine	Thailand
Runchaeron 2017	3898STDY6199573	ERR1218583	Urine	Thailand
Runchaeron 2017	3898STDY6199574	ERR1218584	Sputum	Thailand
Runchaeron 2017	3898STDY6199575	ERR1218585	Urine	Thailand
Runchaeron 2017	3898STDY6199576	ERR1218534	Blood	Thailand
Runchaeron 2017	3898STDY6199577	ERR1218586	Pus	Thailand
Runchaeron 2017	3898STDY6199578	ERR1218587	Blood	Thailand
Runchaeron 2017	3898STDY6199579	ERR1218588	Urine	Thailand

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Runchaeron 2017	3898STDY6199580	ERR1218589	Urine	Thailand
Runchaeron 2017	3898STDY6199581	ERR1218590	Pus	Thailand
Runchaeron 2017	3898STDY6199582	ERR1218591	Pus	Thailand
Runchaeron 2017	3898STDY6199583	ERR1218592	Urine	Thailand
Runchaeron 2017	3898STDY6199584	ERR1218593	Urine	Thailand
Runchaeron 2017	3898STDY6199585	ERR1218594	Urine	Thailand
Runchaeron 2017	3898STDY6199586	ERR1218595	Blood	Thailand
Runchaeron 2017	3898STDY6199587	ERR1218596	Pus	Thailand
Runchaeron 2017	3898STDY6199588	ERR1218597	Urine	Thailand
Runchaeron 2017	3898STDY6199589	ERR1218535	Blood	Thailand
Runchaeron 2017	3898STDY6199590	ERR1218598	Urine	Thailand
Runchaeron 2017	3898STDY6199591	ERR1218599	Pus	Thailand
Runchaeron 2017	3898STDY6199592	ERR1218600	Urine	Thailand
Runchaeron 2017	3898STDY6199593	ERR1218536	Blood	Thailand
Runchaeron 2017	3898STDY6199594	ERR1218601	Urine	Thailand
Runchaeron 2017	3898STDY6199595	ERR1218602	Blood	Thailand
Runchaeron 2017	3898STDY6199596	ERR1218537	Pus	Thailand
Runchaeron 2017	3898STDY6199597	ERR1218538	Pus	Thailand
Runchaeron 2017	3898STDY6199598	ERR1218603	Pus	Thailand
Runchaeron 2017	3898STDY6199599	ERR1218539	Urine	Thailand
Runchaeron 2017	3898STDY6199600	ERR1218604	Pus	Thailand
Runchaeron 2017	3898STDY6199601	ERR1218540	Pus	Thailand
Runchaeron 2017	3898STDY6199602	ERR1218605	Urine	Thailand
Runchaeron 2017	3898STDY6199603	ERR1218606	Urine	Thailand
Runchaeron 2017	3898STDY6199604	ERR1218607	Urine	Thailand
Runchaeron 2017	3898STDY6199605	ERR1218608	Blood	Thailand
Runchaeron 2017	3898STDY6199606	ERR1218609	Pus	Thailand
Runchaeron 2017	3898STDY6199607	ERR1218610	Pus	Thailand
Runchaeron 2017	3898STDY6199608	ERR1218541	Urine	Thailand
Runchaeron 2017	3898STDY6199609	ERR1218611	Blood	Thailand
Runchaeron 2017	3898STDY6199610	ERR1218542	Pus	Thailand
Runchaeron 2017	3898STDY6199611	ERR1218612	Urine	Thailand

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Runchaeron 2017	3898STDY6199612	ERR1218613	Pus	Thailand
Runchaeron 2017	3898STDY6199613	ERR1218614	Blood	Thailand
Runchaeron 2017	3898STDY6199614	ERR1218543	Urine	Thailand
Runchaeron 2017	3898STDY6199615	ERR1218615	Blood	Thailand
Runchaeron 2017	3898STDY6199616	ERR1218616	Urine	Thailand
Runchaeron 2017	3898STDY6199617	ERR1218617	Blood	Thailand
Runchaeron 2017	3898STDY6199618	ERR1218618	Blood	Thailand
Runchaeron 2017	3898STDY6199619	ERR1218619	Urine	Thailand
Runchaeron 2017	3898STDY6199620	ERR1218620	Urine	Thailand
Runchaeron 2017	3898STDY6199621	ERR1218621	Blood	Thailand
Runchaeron 2017	3898STDY6199622	ERR1218544	Urine	Thailand
Runchaeron 2017	3898STDY6199623	ERR1218622	Urine	Thailand
Runchaeron 2017	3898STDY6199624	ERR1218545	Blood	Thailand
Runchaeron 2017	3898STDY6199625	ERR1218623	Urine	Thailand
Runchaeron 2017	3898STDY6199626	ERR1218624	Urine	Thailand
Runchaeron 2017	3898STDY6199627	ERR1218625	Urine	Thailand
Runchaeron 2017	3898STDY6199628	ERR1218626	Urine	Thailand
Runchaeron 2017	3898STDY6199629	ERR1218627	Urine	Thailand
Runchaeron 2017	3898STDY6199630	ERR1218628	Blood	Thailand
Runchaeron 2017	3898STDY6199631	ERR1218629	Blood	Thailand
Runchaeron 2017	3898STDY6199632	ERR1218630	Pus	Thailand
Runchaeron 2017	3898STDY6199633	ERR1218631	Pus	Thailand
Runchaeron 2017	3898STDY6199634	ERR1218632	Urine	Thailand
Runchaeron 2017	3898STDY6199635	ERR1218633	Blood	Thailand
Runchaeron 2017	3898STDY6199636	ERR1218634	Pus	Thailand
Runchaeron 2017	3898STDY6199637	ERR1218635	Urine	Thailand
Runchaeron 2017	3898STDY6199638	ERR1218546	Urine	Thailand
Runchaeron 2017	3898STDY6199639	ERR1218636	Pus	Thailand
Runchaeron 2017	3898STDY6199640	ERR1218637	Urine	Thailand
Runchaeron 2017	3898STDY6199642	ERR1218639	Blood	Thailand
Runchaeron 2017	3898STDY6199643	ERR1218640	Pus	Thailand

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Runchaeron 2017	3898STDY6199644	ERR1218641	Blood	Thailand
Runchaeron 2017	3898STDY6199645	ERR1218642	Canal	Thailand
Runchaeron 2017	3898STDY6199648	ERR1218643	Canal	Thailand
Runchaeron 2017	3898STDY6199649	ERR1218644	Canal	Thailand
Runchaeron 2017	3898STDY6199651	ERR1218549	Canal	Thailand
Runchaeron 2017	3898STDY6199653	ERR1218551	Canal	Thailand
Runchaeron 2017	3898STDY6199654	ERR1218646	Canal	Thailand
Runchaeron 2017	3898STDY6199656	ERR1218552	Canal	Thailand
Runchaeron 2017	3898STDY6199657	ERR1218648	Canal	Thailand
Runchaeron 2017	3898STDY6199658	ERR1218553	Canal	Thailand
Runchaeron 2017	3898STDY6199659	ERR1218649	Canal	Thailand
Runchaeron 2017	3898STDY6199660	ERR1218650	Canal	Thailand
Runchaeron 2017	3898STDY6199661	ERR1218651	Canal	Thailand
Runchaeron 2017	3898STDY6199662	ERR1218652	Canal	Thailand
Runchaeron 2017	3898STDY6199664	ERR1218654	Canal	Thailand
Runchaeron 2017	3898STDY6199665	ERR1218655	Canal	Thailand
Runchaeron 2017	3898STDY6199667	ERR1218656	Canal	Thailand
Runchaeron 2017	3898STDY6199669	ERR1218658	Canal	Thailand
Runchaeron 2017	3898STDY6199670	ERR1218659	Canal	Thailand
Runchaeron 2017	3898STDY6199671	ERR1218660	Canal	Thailand
Runchaeron 2017	3898STDY6199672	ERR1218661	Canal	Thailand
Runchaeron 2017	3898STDY6199673	ERR1218662	Canal	Thailand
Runchaeron 2017	3898STDY6199674	ERR1218663	Canal	Thailand
Runchaeron 2017	3898STDY6199675	ERR1218664	Canal	Thailand
Runchaeron 2017	3898STDY6199677	ERR1218666	Canal	Thailand
Runchaeron 2017	3898STDY6199680	ERR1218669	Canal	Thailand
Runchaeron 2017	3898STDY6199682	ERR1218671	Canal	Thailand
Runchaeron 2017	3898STDY6199685	ERR1218674	Canal	Thailand
Runchaeron 2017	3898STDY6199686	ERR1218675	Canal	Thailand
Runchaeron 2017	3898STDY6199687	ERR1218676	Canal	Thailand
Runchaeron 2017	3898STDY6199689	ERR1218678	Canal	Thailand
Runchaeron 2017	3898STDY6199692	ERR1218681	Canal	Thailand

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Runchaeron 2017	3898STDY6199693	ERR1218682	Canal	Thailand
Runchaeron 2017	3898STDY6199694	ERR1218683	Canal	Thailand
Runchaeron 2017	3898STDY6199695	ERR1218684	Canal	Thailand
Runchaeron 2017	3898STDY6199696	ERR1218685	Canal	Thailand
Runchaeron 2017	3898STDY6199697	ERR1218686	Canal	Thailand
Runchaeron 2017	3898STDY6199698	ERR1218554	Canal	Thailand
Runchaeron 2017	3898STDY6199700	ERR1218688	Canal	Thailand
Runchaeron 2017	3898STDY6199701	ERR1218689	Canal	Thailand
Runchaeron 2017	3898STDY6199702	ERR1218690	Untreated hospital sewage	Thailand
Runchaeron 2017	3898STDY6199704	ERR1218692	Untreated hospital sewage	Thailand
Runchaeron 2017	3898STDY6199705	ERR1218693	Untreated hospital sewage	Thailand
Runchaeron 2017	3898STDY6199706	ERR1218694	Canal	Thailand
Runchaeron 2017	3898STDY6199707	ERR1218695	Canal	Thailand
Runchaeron 2017	3898STDY6199708	ERR1218696	Canal	Thailand
Runchaeron 2017	3898STDY6199709	ERR1218697	Farm	Thailand
Runchaeron 2017	3898STDY6199710	ERR1218698	Farm	Thailand
Runchaeron 2017	3898STDY6199713	ERR1218701	Farm	Thailand
Runchaeron 2017	3898STDY6199714	ERR1218702	Farm	Thailand
Runchaeron 2017	3898STDY6199715	ERR1218703	Farm	Thailand
Runchaeron 2017	3898STDY6199764	ERR1218705	Urine	Thailand
Runchaeron 2017	3898STDY6199766	ERR1218556	Pus	Thailand
Runchaeron 2017	3898STDY6199768	ERR1218706	Urine	Thailand
Runchaeron 2017	3898STDY6199769	ERR1218707	Urine	Thailand
Runchaeron 2017	3898STDY6199772	ERR1218708	Pus	Thailand
Runchaeron 2017	3898STDY6199773	ERR1218557	Pus	Thailand
Runchaeron 2017	3898STDY6199778	ERR1218709	Canal	Thailand
Runchaeron 2017	3898STDY6199780	ERR1218710	Canal	Thailand
Runchaeron 2017	3898STDY6199781	ERR1218558	Canal	Thailand
Runchaeron 2017	3898STDY6199784	ERR1218711	Canal	Thailand
Runchaeron 2017	3898STDY6199790	ERR1218559	Urine	Thailand
Runchaeron 2017	3898STDY6199792	ERR1218712	Blood	Thailand
Runchaeron 2017	3898STDY6199793	ERR1218713	Urine	Thailand

Table 7.1: Details of samples included in global phylogeny (*continued*)

Study	Sample ID	Accession No.	Source	Country
Runchaeron 2017	3898STDY6199796	ERR1218560	Pus	Thailand
Runchaeron 2017	3898STDY6199798	ERR1218714	Blood	Thailand
Runchaeron 2017	3898STDY6199799	ERR1218715	Canal	Thailand
Runchaeron 2017	3898STDY6199802	ERR1218716	Canal	Thailand
Runchaeron 2017	3898STDY6199804	ERR1218561	Canal	Thailand
Runchaeron 2017	3898STDY6199805	ERR1218717	Canal	Thailand
Runchaeron 2017	3898STDY6199806	ERR1218718	Canal	Thailand
Runchaeron 2017	3898STDY6199807	ERR1218719	Canal	Thailand
Runchaeron 2017	3898STDY6199808	ERR1218562	Canal	Thailand
Runchaeron 2017	3898STDY6199809	ERR1218720	Canal	Thailand
Runchaeron 2017	3898STDY6199815	ERR1218564	Farm	Thailand
Runchaeron 2017	3898STDY6199816	ERR1218723	Canal	Thailand
Runchaeron 2017	3898STDY6199923	ERR1218774	Canal	Thailand

Note:

RS = Rectal swab

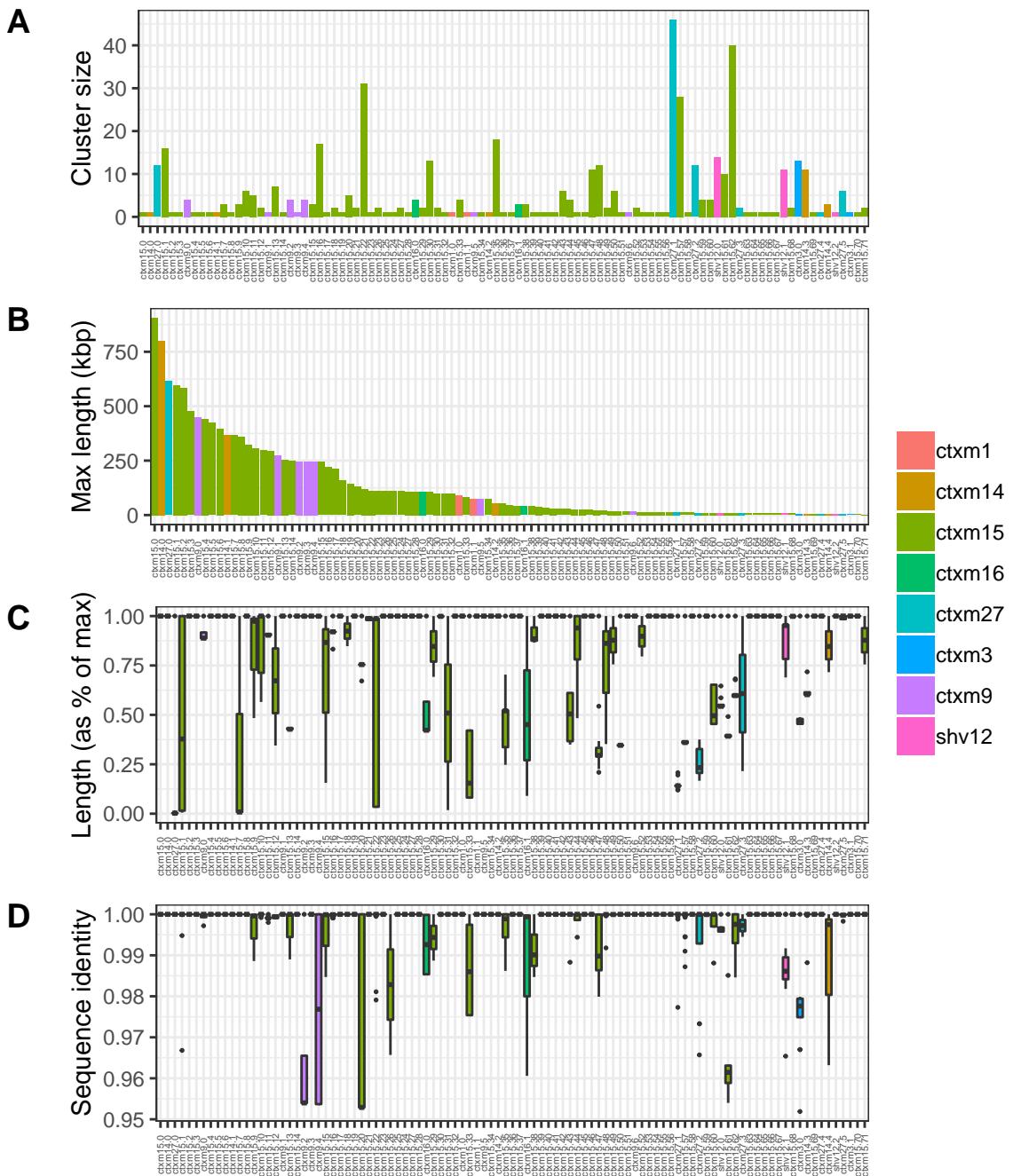


Figure 7.17: Summary statistics for 99 ESBL-containing contig clusters as determined by *cd-hit*. A: Number of contigs per cluster. B: Length (kbp) of longest sample in each cluster. This is defined as the cluster representative sample by *cd-hit* to which all other samples are compared for the purposes of length and sequence identity. C: Distribution of contig lengths by cluster expressed as a proportion of longest contig length. D: Distribution of sequence identity of cluster members compared to representative member, by cluster.



Figure 7.18: AMR genes, insertion sequences (IS) and plasmid replicons identified in the representative contig of each contig cluster, stratified by ESBL gene and ordered by number of samples of cluster. IS26 is very frequently associated with a 108bp fragment of *catB4* chloramphenicol resistance gene, shown as a red fragment within the green IS26 element. A: *blaCTXM15*, B: *blaCTXM27*, C: *blaSHV12*. Plots show furthest IS/AMR gene or plasmid replicon up to +/- 10,000bp from the ESBL gene of interest.

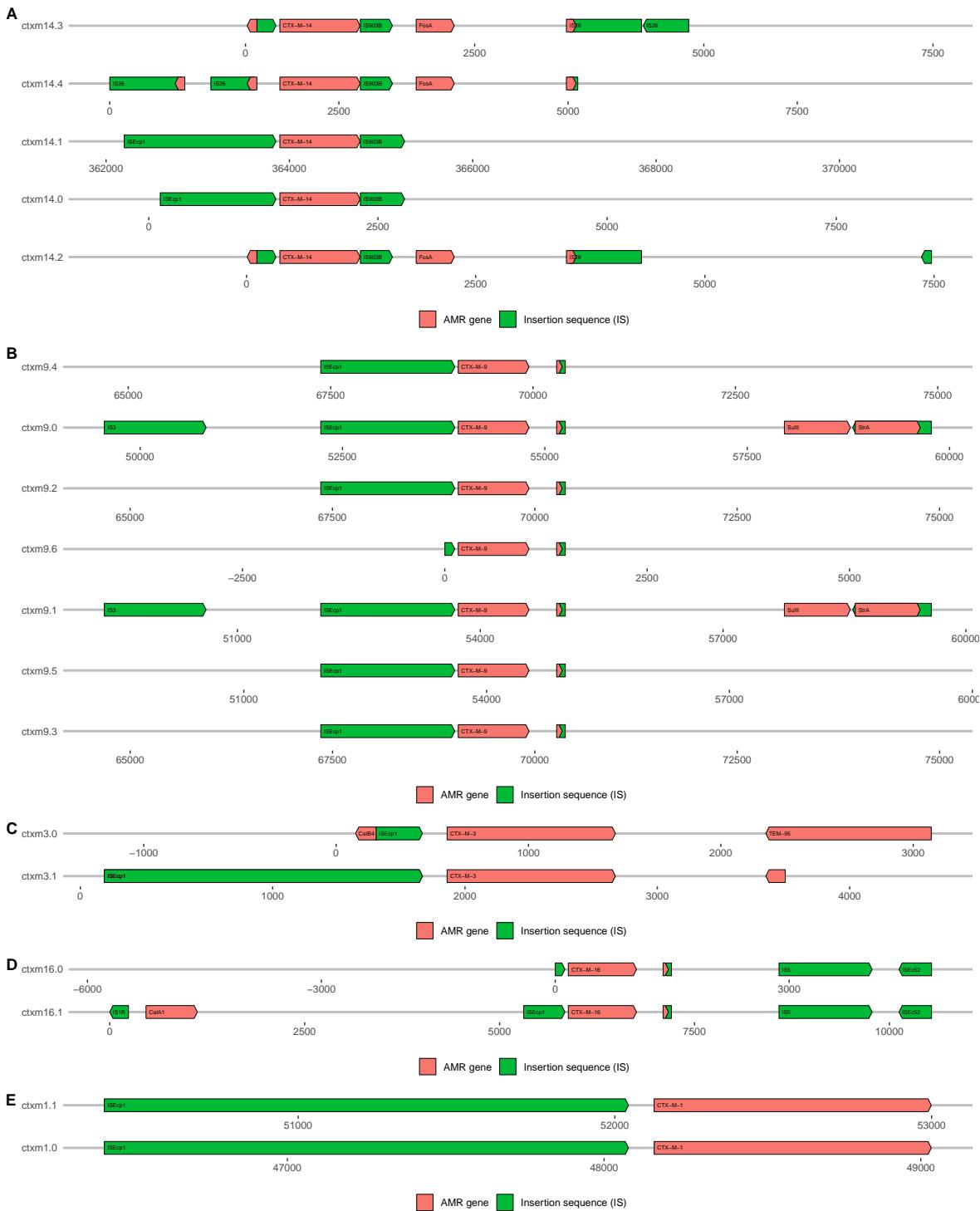


Figure 7.19: AMR genes, insertion sequences (IS) and plasmid replicons identified in the representative contig of each contig cluster, stratified by by ESBL gene and ordered by number of samples in cluster. IS26 is very frequently associated with a 108bp fragment of *catB4* chloramphenicol resistance gene, shown as a red fragnemt within the green IS26 element. A: *blaCTXM14*, B: *blaCTXM9* , C: *blaCTXM3*, D: *blaCTXM16*, E: *blaCTXM1*. Plots show furthest IS/AMR gene or plasmid replicon up to +/- 10,000bp from the ESBL gene of interest.

References

- 1 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
- 2 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
- 3 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
- 4 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
- 5 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>
- 6 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
- 7 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
- 8 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

- 9 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
- 10 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
- 11 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
- 12 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
- 13 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
- 14 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
- 15 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
- 16 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
- 17 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
- 18 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
- 19 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
- 20 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
- 21 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
- 22 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
- 23 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
- 24 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
- 25 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
- 26 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
- 27 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
- 28 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
- 29 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
- 30 Mourembou G, Nzondo SM, Ndjoyi-Mbiquino 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

- 31 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
- 32 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
- 33 Sothmann P, Keller C, Krumkamp R *et al.* <i>Rickettsia felis</i> Infection in Febrile Children, Ghana. *The American Journal of Tropical Medicine and Hygiene* 2017;96:16–0754. doi:10.4269/ajtmh.16-0754
- 34 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
- 35 Mourembou G, Lekana-Douki JB, Mediannikov O *et al.* Possible Role of <i>Rickettsia felis</i> in Acute Febrile Illness among Children in Gabon. *Emerging Infectious Diseases* 2015;21:1808–15. doi:10.3201/eid2110.141825
- 36 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
- 37 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
- 38 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
- 39 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
- 40 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
- 41 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

- 42 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
- 43 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
- 44 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
- 45 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
- 46 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
- 47 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
- 48 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
- 49 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
- 50 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
- 51 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
- 52 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
- 53 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>
- 54 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
- 55 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
- 56 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>
- 57 Seymour CW, Liu VX, Iwashyna TJ *et al.* Assessment of Clinical Criteria for Sepsis. *JAMA* 2016;315:762. doi:10.1001/jama.2016.0288
- 58 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
- 59 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
- 60 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
- 61 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
- 62 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
- 63 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
- 64 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

- 65 Gaieski DF, Edwards JM, Kallan MJ *et al.* Benchmarking the Incidence and Mortality of Severe Sepsis in the United States*. *Critical Care Medicine* 2013;41:1167–74. doi:10.1097/CCM.0b013e31827c09f8
- 66 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
- 67 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>
- 68 Tservadze 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
- 69 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
- 70 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
- 71 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
- 72 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
- 73 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
- 74 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
- 75 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
- 76 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

77 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

78 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

79 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

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

81 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

82 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

83 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

84 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

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

86 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

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

- 88 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
- 89 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
- 90 Onishi A, Sugiyama D, Kogata Y *et al.* Diagnostic Accuracy of Serum 1,3- β -d-Glucan for Pneumocystis jiroveci Pneumonia, Invasive Candidiasis, and Invasive Aspergillosis: Systematic Review and Meta-Analysis. *Journal of Clinical Microbiology* 2012;50:7–15. doi:10.1128/JCM.05267-11
- 91 Rhodes A, Evans LE, Alhazzani W *et al.* Surviving Sepsis Campaign. *Critical Care Medicine* 2017;45:486–552. doi:10.1097/CCM.0000000000002255
- 92 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
- 93 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
- 94 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
- 95 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
- 96 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
- 97 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
- 98 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

- 99 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
- 100 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
- 101 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
- 102 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
- 103 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
- 104 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
- 105 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
- 106 Leisman D, Wie B, Doerfler M *et al.* Association of Fluid Resuscitation Initiation Within 30 Minutes of Severe Sepsis and Septic Shock Recognition With Reduced Mortality and Length of Stay. *Annals of Emergency Medicine* 2016;68:298–311. doi:10.1016/j.annemergmed.2016.02.044
- 107 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
- 108 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
- 109 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

110 Maitland K, George EC, Evans JA *et al.* Exploring mechanisms of excess mortality with early fluid resuscitation: insights from the FEAST trial. *BMC Medicine* 2013;11:68. doi:10.1186/1741-7015-11-68

111 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

112 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

113 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

114 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

115 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

116 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

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

118 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

119 Agyekum A, Fajardo-Lubian A, Ansorg 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
- 120 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
- 121 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
- 122 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
- 123 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
- 124 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
- 125 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
- 126 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
- 127 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
- 128 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
- 129 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
- 130 Sangare SA, Maiga AI, Guindo I *et al.* Prevalence of ESBL-producing Enterobac-

- teriaceae isolated from blood cultures in Mali. *J Infect Dev Ctries* 2016;10:1059–64. doi:10.3855/jidc.7536
- 131 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
- 132 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
- 133 Irenege 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
- 134 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
- 135 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
- 136 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
- 137 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
- 138 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
- 139 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
- 140 Irenege 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

- 141 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
- 142 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>
- 143 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
- 144 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
- 145 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
- 146 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
- 147 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
- 148 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
- 149 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
- 150 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
- 151 Bercion R, Mossoro-Kpinde D, Manirakiza A *et al.* Increasing prevalence of antimicrobial resistance among Enterobacteriaceae uropathogens in Bangui, Central African Republic. *J In-*

- fect Dev Ctries 2009;3:187–90.https://jidc.org/index.php/journal/article/download/19759473/21
- 152 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
- 153 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
- 154 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
- 155 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
- 156 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
- 157 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
- 158 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
- 159 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
- 160 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
- 161 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
- 162 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

163 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

164 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

165 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

166 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

167 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

168 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

169 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

170 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

171 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

172 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

173 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

174 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

175 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

176 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

177 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

178 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

179 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

180 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

181 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

182 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

183 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

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

185 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

186 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

187 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

188 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

189 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

190 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

191 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

192 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

- 193 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
- 194 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
- 195 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
- 196 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
- 197 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
- 198 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
- 199 Bush K, Jacoby GA. Updated functional classification of beta-lactamases. *Antimicrobial agents and chemotherapy* 2010;54:969–76. doi:10.1128/AAC.01009-09
- 200 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
- 201 ABRAHAM EP, CHAIN E. An Enzyme from Bacteria able to Destroy Penicillin. *Nature* 1940;146:837–7. doi:10.1038/146837a0
- 202 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>
- 203 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>
- 204 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.ncbi.nlm.nih.gov/pubmedcentral.nih.gov/articlerender.fcgi?artid=PA1326330>

- 205 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
- 206 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>
- 207 Gold HS, Moellering RC. Antimicrobial-Drug Resistance. *New England Journal of Medicine* 1996;335:1445–53. doi:10.1056/NEJM199611073351907
- 208 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>
- 209 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
- 210 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
- 211 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
- 212 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>
- 213 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
- 214 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
- 215 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
- 216 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

- 217 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
- 218 Mirelis B, Navarro F, Miró E *et al.* Community Transmission of Extended-Spectrum β -Lactamase. *Emerging Infectious Diseases* 2003;9:1024–5. doi:10.3201/eid0908.030094
- 219 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
- 220 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
- 221 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
- 222 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
- 223 Ny S, Löfmark S, Börjesson S *et al.* Community carriage of ESBL-producing <i>Escherichia coli</i> is associated with strains of low pathogenicity: a Swedish nationwide study. *Journal of Antimicrobial Chemotherapy* 2017;72:582–8. doi:10.1093/jac/dkw419
- 224 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
- 225 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
- 226 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
- 227 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*

- apy 2016;71:1076–82. doi:10.1093/jac/dkv441
- 228 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
- 229 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
- 230 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
- 231 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
- 232 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
- 233 Zahar J, Lanterrier F, Mechali 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
- 234 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
- 235 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
- 236 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
- 237 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

- 238 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
- 239 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
- 240 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
- 241 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
- 242 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
- 243 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
- 244 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
- 245 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
- 246 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
- 247 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
- 248 National Statistics Office of Malawi. Malawi population projections. www.nsomalawi.mw (accessed 10 Feb 2019).
- 249 World Bank. World Bank Country and Lending Groups. <https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups> (accessed 28 Feb 2019).

- 250 United Nations Development Programme. Human Development Indices and Indicators: 2018 Statistical Update. 2018.
- 251 UNAIDS. Malawi Country Profile. <http://aidsinfo.unaids.org/> (accessed 28 Feb 2019).
- 252 British Society for Antimicrobial Chemotherapy. BSAC Methods for Antimicrobial Susceptibility Testing. 2013.
- 253 Heller T, Wallrauch C, Goblirsch S *et al.* Focused assessment with sonography for HIV-associated tuberculosis (FASH): a short protocol and a pictorial review. *Critical ultrasound journal* 2012;4:21. doi:10.1186/2036-7902-4-21
- 254 Ministry of Health. Government of Malawi. Malawi HIV Testing Service Guidelines. 2015.
- 255 Wood DE, Salzberg SL. Kraken: ultrafast metagenomic sequence classification using exact alignments. *Genome Biology* 2014;15:R46. doi:10.1186/gb-2014-15-3-r46
- 256 Bankevich A, Nurk S, Antipov D *et al.* SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *Journal of Computational Biology* 2012;19:455–77. doi:10.1089/cmb.2012.0021
- 257 Gurevich A, Saveliev V, Vyahhi N *et al.* QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 2013;29:1072–5. doi:10.1093/bioinformatics/btt086
- 258 Parks DH, Imelfort M, Skennerton CT *et al.* CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome research* 2015;25:1043–55. doi:10.1101/gr.186072.114
- 259 Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 2014;30:2068–9. doi:10.1093/bioinformatics/btu153
- 260 Page AJ, Cummins CA, Hunt M *et al.* Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics* 2015;31:3691–3. doi:10.1093/bioinformatics/btv421
- 261 Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular biology and evolution* 2013;30:772–80. doi:10.1093/molbev/mst010
- 262 Page AJ, Taylor B, Delaney AJ *et al.* SNP-sites: rapid efficient extraction of SNPs from multi-FASTA alignments. *Microbial Genomics* 2016;2. doi:10.1099/mgen.0.000056
- 263 Nguyen L-T, Schmidt HA, Haeseler A von *et al.* IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Molecular Biology and Evolution* 2015;32:268–74. doi:10.1093/molbev/msu300

- 264 Yu G, Smith DK, Zhu H *et al.* Ggtree : An r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods in Ecology and Evolution* 2017;8:28–36. doi:10.1111/2041-210X.12628
- 265 Hunt M, Mather AE, Sánchez-Busó L *et al.* ARIBA: rapid antimicrobial resistance genotyping directly from sequencing reads. *Microbial genomics* 2017;3:e000131. doi:10.1099/mgen.0.000131
- 266 Inouye M, Dashnow H, Raven L-A *et al.* SRST2: Rapid genomic surveillance for public health and hospital microbiology labs. *Genome Medicine* 2014;6:90. doi:10.1186/s13073-014-0090-6
- 267 Carattoli A, Zankari E, García-Fernández A *et al.* In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrobial agents and chemotherapy* 2014;58:3895–903. doi:10.1128/AAC.02412-14
- 268 Clermont O, Christenson JK, Denamur E *et al.* The Clermont <i>Escherichia coli</i> phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. *Environmental Microbiology Reports* 2013;5:58–65. doi:10.1111/1758-2229.12019
- 269 Cheng L, Connor TR, Siren J *et al.* Hierarchical and Spatially Explicit Clustering of DNA Sequences with BAPS Software. *Molecular Biology and Evolution* 2013;30:1224–8. doi:10.1093/molbev/mst028
- 270 Altschul SF, Gish W, Miller W *et al.* Basic local alignment search tool. *Journal of Molecular Biology* 1990;215:403–10. doi:10.1016/S0022-2836(05)80360-2
- 271 Li W, Godzik A. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* 2006;22:1658–9. doi:10.1093/bioinformatics/btl158
- 272 Runcharoen C, Raven KE, Reuter S *et al.* Whole genome sequencing of ESBL-producing *Escherichia coli* isolated from patients, farm waste and canals in Thailand. *Genome Medicine* 2017;9:81. doi:10.1186/s13073-017-0471-8
- 273 Mentzer A von, Connor TR, Wieler LH *et al.* Identification of enterotoxigenic *Escherichia coli* (ETEC) clades with long-term global distribution. *Nature Genetics* 2014;46:1321–6. doi:10.1038/ng.3145
- 274 Ingle DJ, Tauschek M, Edwards DJ *et al.* Evolution of atypical enteropathogenic *E. coli* by repeated acquisition of LEE pathogenicity island variants. *Nature Microbiology* 2016;1:15010. doi:10.1038/nmicrobiol.2015.10
- 275 Musicha P, Feasey NA, Cain AK *et al.* Genomic landscape of extended-spectrum beta-lactamase resistance in *Escherichia coli* from an urban African setting. *J Antimicrob Chemother*

2017;72:1602–9. doi:10.1093/jac/dkx058

276 Dale AP, Woodford N. Extra-intestinal pathogenic Escherichia coli (ExPEC): Disease, carriage and clones. *Journal of Infection* 2015;71:615–26. doi:10.1016/J.JINF.2015.09.009

277 Krishnaraju M, Kamatchi C, Jha AK *et al.* Complete sequencing of an IncX3 plasmid carrying blaNDM-5 allele reveals an early stage in the dissemination of the blaNDM gene. *Indian journal of medical microbiology* 2015;33:30–8. doi:10.4103/0255-0857.148373

278 Ostrer L, Khodursky RF, Johnson JR *et al.* Analysis of mutational patterns in quinolone resistance-determining regions of Gyra and ParC of clinical isolates. *International Journal of Antimicrobial Agents* 2019;53:318–24. doi:10.1016/j.ijantimicag.2018.12.004

279 Ramirez MS, Tolmasky ME. Aminoglycoside modifying enzymes. *Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy* 2010;13:151–71. doi:10.1016/j.drup.2010.08.003

280 Galimand M, Courvalin P, Lambert T. Plasmid-Mediated High-Level Resistance to Aminoglycosides in Enterobacteriaceae Due to 16S rRNA Methylation. *Antimicrobial Agents and Chemotherapy* 2003;47:2565. doi:10.1128/AAC.47.8.2565-2571.2003

281 Anantham S, Hall RM. pCERC1, a Small, Globally Disseminated Plasmid Carrying the <i>dfrA14</i> Cassette in the <i>strA</i> Gene of the <i>sul2-strA-strB</i> Gene Cluster. *Microbial Drug Resistance* 2012;18:364–71. doi:10.1089/mdr.2012.0008

282 Pitout JDD, DeVinney R. Escherichia coli ST131: a multidrug-resistant clone primed for global domination. *F1000Research* 2017;6:195. doi:10.12688/f1000research.10609.1

283 Roer L, Overballe-Petersen S, Hansen F *et al.* <i>Escherichia coli</i> Sequence Type 410 Is Causing New International High-Risk Clones. *mSphere* 2018;3:e00337–18. doi:10.1128/mSphere.00337-18

284 Zong Z, Fenn S, Connor C *et al.* Complete genomic characterization of two Escherichia coli lineages responsible for a cluster of carbapenem-resistant infections in a Chinese hospital. *Journal of Antimicrobial Chemotherapy* 2018;73:2340–6. doi:10.1093/jac/dky210

285 Wang Q, Wang X, Wang J *et al.* Phenotypic and Genotypic Characterization of Carbapenem-resistant Enterobacteriaceae : Data From a Longitudinal Large-scale CRE Study in China (2012–2016). *Clinical Infectious Diseases* 2018;67:S196–205. doi:10.1093/cid/ciy660

286 Irrgang A, Falgenhauer L, Fischer J *et al.* CTX-M-15-Producing E. coli Isolates from Food Products in Germany Are Mainly Associated with an IncF-Type Plasmid and Belong to Two Predominant Clonal E. coli Lineages. *Frontiers in Microbiology* 2017;8:2318. doi:10.3389/fmicb.2017.02318

- 287 Kallonen T, Brodrick HJ, Harris SR *et al.* Systematic longitudinal survey of invasive < i > Escherichia coli < /i > in England demonstrates a stable population structure only transiently disturbed by the emergence of ST131. *Genome Research* 2017; **27**:1437–49. doi:10.1101/gr.216606.116
- 288 Malawi Ministry of Health. Malawi Guidelines for Clinical Management of HIV in Children and Adults (Third Edition). 2016.
- 289 Neuert S, Nair S, Day MR *et al.* Prediction of Phenotypic Antimicrobial Resistance Profiles From Whole Genome Sequences of Non-typhoidal *Salmonella enterica*. *Frontiers in microbiology* 2018; **9**:592. doi:10.3389/fmicb.2018.00592
- 290 Williams CT, Musicha P, Feasey NA *et al.* ChloS-HRM, a novel assay to identify chloramphenicol-susceptible *Escherichia coli* and *Klebsiella pneumoniae* in Malawi. *The Journal of antimicrobial chemotherapy* 2019; **74**:1212. doi:10.1093/jac/dky563
- 291 Totsika M, Beatson SA, Sarkar S *et al.* Insights into a Multidrug Resistant *Escherichia coli* Pathogen of the Globally Disseminated ST131 Lineage: Genome Analysis and Virulence Mechanisms. *PLoS ONE* 2011; **6**:e26578. doi:10.1371/JOURNAL.PONE.0026578
- 292 Stoesser N, Sheppard AE, Moore CE *et al.* Extensive Within-Host Diversity in Frequently Carried Extended-Spectrum-Beta-Lactamase-Producing *Escherichia coli* Isolates: Implications for Transmission Analyses. *Journal of clinical microbiology* 2015; **53**:2122–31. doi:10.1128/JCM.00378-15