Emerging resistance to empiric antimicrobial regimens for pediatric bloodstream infections in Malawi (1998-2017)

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Summary

Incidence of bloodstream infections in Malawian children declined significantly over two decades, but resistance of Gram-negative pathogens to empiric first-line antimicrobials increased from 3.4% to 30.2% for children ≤5 years and 7.0% to 67.7% for young infants ≤60 days.

Abstract

Background: The adequacy of the WHO Integrated Management of Childhood Illness (IMCI) antimicrobial guidelines for the treatment of suspected severe bacterial infections is dependent on a low prevalence of antimicrobial resistance (AMR). We describe trends in etiologies and susceptibility patterns of bloodstream infections (BSI) in hospitalized children in Malawi.

Methods: We determined the change in population-based incidence of BSI in children admitted to Queen Elizabeth Central Hospital, Blantyre, Malawi (1998-2017). AMR profiles were assessed by the disc diffusion method and trends over time were evaluated.

Results: A total 89,643 pediatric blood cultures were performed, and 10,621 pathogens were included in the analysis. Estimated minimum incidence rates of BSI for those ≤5 years of age fell from a peak of 11.4 per 1,000 persons in 2002 to 3.4 per 1,000 persons in 2017. Over two decades, resistance of Gram-negative pathogens to all empiric first-line antimicrobials (ampicillin/penicillin, gentamicin, ceftriaxone) among children ≤5 years increased from 3.4% to 30.2% ($p<0.001$). Among those ≤60 days, AMR to all first-line antimicrobials increased from 7.0% to 67.7% ($p<0.001$). Among children ≤5 years, *Klebsiella* spp. resistance to all first-line antimicrobial regimens increased from 5.9% to 93.7% ($p<0.001$).

Conclusions: The incidence of BSI among hospitalized children has decreased substantially over the last 20 years, although gains have been offset by increases in Gram-negative pathogens resistant to all empiric first-line antimicrobials. There is an urgent need to address the broader challenge of adapting IMCI guidelines to the local setting in the face of rapidly expanding AMR in childhood BSI.

Keywords: Antimicrobial resistance; pediatric; neonatal; sepsis; Gram negative

Sepsis accounted for half a million child deaths globally in 2000, and had the lowest decline of all the top seven causes of death in this population by 2015, with only a 25% decrease compared to other leading causes such as pneumonia (47%), diarrhea (57%) and measles (85%) [1]. In 2015, sepsis and other infectious conditions of the newborn accounted for 7% of all deaths worldwide among children under 5 years of age [1]. Empiric first-line antimicrobial treatment for sick infants in the World Health Organization (WHO) Integrated Management of Childhood Illness (IMCI) guidelines consists of penicillin/ampicillin with gentamicin, or ceftriaxone (as is practiced in Malawi). At our center in Malawi, ceftriaxone has been available since 2001, was introduced as an empiric antibiotic for neonatal meningitis in 2009 [2], and has been used as first-line treatment for suspected neonatal sepsis since 2013 [3]. Pediatric departmental guidelines recommend use of penicillin and gentamicin for suspected sepsis, or ceftriaxone for suspected meningitis, typhoid fever and non-typhoidal *Salmonella* (NTS). These recommendations are based on sparse data from low- and middle-income countries (LMICs), and are intended to be adjusted to local susceptibility patterns so that 'appropriate therapy [can be given] for an identified bacterial cause' [4]. This can be challenging in LMICs, where paucity of diagnostic microbiology facilities [5] limits the ability of clinicians to optimize empiric therapy.

Child mortality in Malawi has been falling in the last 20 years as a consequence of multiple interventions including better nutrition, new vaccines (*H. influenzae* type b conjugate vaccine in 2002, and *S. pneumoniae* conjugate vaccine in 2011), rapid roll-out of HIV prevention and care and improvements in malaria control [6-11]. A growing problem has been increasing rates of antimicrobial resistance (AMR) [12, 13], which may be caused and compounded by inappropriate prescribing, under-dosing, and

nonadherence to guidelines [14, 15]. There is a scarcity of high quality bloodstream infection (BSI) surveillance data to inform policy change. We have previously documented the expansion of extended-spectrum beta-lactamase (ESBL) and fluoroquinolone resistance among common Gram-negative pathogens, as well as the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in BSI [12], but did not explore this in detail in the population ≤5 years where the impact may be greatest. This study reviews the pediatric surveillance data collected over two decades at an urban district hospital and tertiary referral center in Malawi. We describe trends in the etiology and the prevalence of AMR amongst BSI isolates cultured from hospitalized children. Finally, we assess the adequacy of current IMCI guidelines in this setting.

Methods

Setting

Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi, is a 1,250 bed government-funded teaching hospital providing free medical care and is the main teaching hospital of the University of Malawi College of Medicine. It is the referral center for the southern half of the country and serves as the district hospital for the urban Blantyre area (estimated population 920,000 in 2016). The pediatric department admits 20,000-30,000 children a year, with 65,000-80,000 seen annually in the pediatric Accident and Emergency unit. Total numbers of pediatric hospital admissions have remained broadly constant over the study period (Supplementary Table 1). Similarly, the neonatal unit admits 3,500 neonates a year, and these numbers have remained consistent over the past decade, when admission data were first collected.

Study design

We reviewed blood culture data collected at QECH over 20 consecutive years (1998-2017; Supplementary Figure 1). Blood culture sampling in children was obtained from any pediatric patient with: clinical suspicion of sepsis, severe sepsis or septic shock [16]; non-focal febrile illness who tested negative for malaria, or who remained febrile despite empiric antimicrobial treatment. Sampling followed set departmental guidelines which have remained unchanged over the period of surveillance. Repeat blood cultures were not routinely done in the setting of a positive result. Precise individual or population-based antimicrobial usage data for the surveillance period were not available. Analysis of microbiological surveillance data was approved by the University of Malawi College of Medicine Research Ethics Committee (COMREC, P.08/14/1614).

Microbiology

Routine, quality-assured, diagnostic blood culture service has been provided for children admitted to QECH by the Malawi-Liverpool-Wellcome Trust Clinical Research Programme since 1998. Among children, 1-2 mLs of blood were obtained where possible for culture under aseptic conditions and inoculated into a single aerobic bottle (BacT/Alert, bioMérieux, Marcy-L'Etoile, France). All lab data over the study period were collected for children ≤5 years, including young infants <60 days. Further clinical data was unavailable. Where possible, all duplicates were removed.

The automated BacT/Alert system (bioMérieux, France) has been used to incubate samples since 2000. Before then, manual culture was used as previously described [17] with organism identification using APIs (Biomérieux). Staphylococci were identified by slide coagulase, beta-hemolytic Streptococci by Lancefield antigen testing and Salmonellae by serotyping according to the White-Kauffmann-Le Minor

scheme. *Haemophilus influenzae* were typed using type b antisera. *Aerococcus* spp., *Alcaligenes* spp., alpha-hemolytic streptococci (other than *S. pneumoniae*), *Bacillus* spp., Corynebacteria, *Micrococcus* spp., coagulase negative staphylococci, unidentified gram-positive rods, and *Rhizobium* spp. that form part of the normal skin or oral flora, were considered contaminants.

In Malawi, first-line antimicrobial regimens for treatment of pediatric and neonatal BSI include ampicillin/penicillin with gentamicin, or ceftriaxone. Antimicrobial susceptibility was determined by the disc diffusion method (Oxoid, United Kingdom) following the current version of the British Society of Antimicrobial Chemotherapy guidelines (BSAC, http://www.bsac.org.uk). Intermediate susceptibility (with the exception of *S. pneumoniae*) was regarded as resistant. Methicillin resistance in *S. aureus* was inferred by resistance to cefoxitin, which replaced oxacillin resistance testing in 2010. For *S. pneumoniae*, reduced susceptibility to penicillin was detected by oxacillin disc, with resistance defined as MIC >2 µg/mL. Formal MIC testing was not done. Since 2007, gram-negative isolates have been screened for ESBL-producing status using a cefpodoxime disc. Prior to this, ESBL-producing status was inferred based on resistance to ceftriaxone.

Statistical analysis

We estimated minimum annual incidence rates, expressed as incidence per 1,000 age-specific person years, by dividing the number of bacteremia cases per year by mid-year population and multiplying by 1,000. We modeled the observed annual case frequencies and then estimated incidence by dividing the predicted case frequencies by the mid-year populations. Age-stratified population estimates for urban Blantyre for the years 1998-2017 were obtained from the 1998 and 2008 National Population Projections by the National Statistical Office (NSO, http://www.nsomalawi.mw). We used yearly values for children ≤5 years, but when there were low numbers of cases including for young infants ≤60 days, we combined

data into 5-year periods to enable comparisons. We followed the WHO definition of young infants as ≤60 days [4] and defined early-onset neonatal BSI as <7 days and late-onset neonatal BSI as 7-90 days. We used negative binomial regression models to test for linear trend in incidence and the Cochran-Armitage test to detect trends in AMR rates. All statistical analyses were performed using R Statistical Package version 3.3.2 for MacOS (R Core Team, http://www.r-project.org).

Results

Between 1998-2017, a total of 89,643 blood cultures from pediatric patients were identified. Of these, 10,621 pathogenic bacteria were identified from children ≤5 years, including 2,898 from young infants ≤60 days.

Incidence rates

Minimum incidence rates of pediatric BSI decreased significantly over two decades (Figure 1), falling from a peak of 11.4 per 1,000 persons in 2002 for children ≤5 years to 3.4 per 1,000 persons in 2017 (overall decreasing trend, $p<0.001$). For young infants, minimum incidence rates decreased from 8.7 per 1,000 persons in 2000 to 1.7 per 1,000 persons in 2008 with an overall decreasing trend of incidence ($p=0.05$). However, since 2008, minimum incidence rates for young infants have been rising, with a steep rise from 2.8 per 1,000 persons in 2015 to 6.3 per 1,000 persons in 2017. For most pathogens there is an overall decreasing trend in minimum incidence rates (Figures 1B-F) over the two decades, with the exception of *Salmonella* Typhi and *Klebsiella* spp. For *S.* Typhi, minimum incidence rates for children ≤5 years increased from a low of 0 per 1,000 persons in 2003 to a peak of 1.3 per 1,000 persons in 2013, and then declined to 0.3 per 1,000 persons in 2017 (overall increasing trend, $p=0.0016$); for *Klebsiella* spp., minimum incidence rates increased from a low of 0.2 per 1,000 persons in 2012 to 0.9 per 1,000 persons

in 2017 (p=0.727). However, when the outlier values for 2016 and 2017 were excluded, there was a declining trend for *Klebsiella* spp. sepsis between 1998 and 2015. (p=0.009)

Pathogen etiology

During the first period (1998-2002; Table 1), NTS accounted for 41.4% (1,644/3,964) of pathogenic isolates, followed by *S. pneumoniae* at 10.2% (405/3,964), and *S. aureus* and *E. coli* at 6.2% each (247/3,964). In the last period (2013-2017), the most common causes of pediatric BSI were *Salmonella* Typhi (544/2,614, 20.8%), *Klebsiella* spp. (316/2,614, 12.1%, with *K. pneumoniae* comprising 93.7% (295) of these), NTS (303/2,614, 11.6%), and *Staphylococcus aureus* (253/2,614, 9.7%). *S. pneumoniae* accounted for 1.9% (49/2,614) of isolates in the last period. For young infant BSI in the first period (Supplementary Table 2), the most common causes were NTS (133/835, 15.9%), *S. aureus* (118/835, 14.1%), and Group B Strep (GBS, 109/835, 13.1%). In the last period, the most common causes were *Klebsiella* spp. (230/800, 28.9%), followed by *S. aureus* (139/800, 17.4%), *Enterobacter* spp. (101/800, 12.6%), *Enterococcus* spp. (72/800, 9%), *E. coli* (57/800, 7.1%) and GBS (43/800, 5.4%). Only 0.6% (5/800) of *S. pneumoniae* specimens were isolated from young infants in the last period compared to 6.2% (52/835) in the first.

AMR profiles

For Gram-positive pathogens, resistance to empiric first-line antimicrobials (ampicillin/penicillin with gentamicin, or ceftriaxone) was 21.1% (605/2,863) and 6.2% (74/1,199) of isolates, respectively. For Gram-negative pathogens, the proportion of culture-confirmed BSI among children ≤5 years that were resistant to all first-line antimicrobials had an overall increasing trend over time, from 3.4% (8/235) in the first period to 30.2% (449/1,487) in the last period (p<0.001; Figure 2). For young infants, the overall

proportion of Gram-negative bacteria resistant to all first-line antimicrobials increased from 7.0% (3/43) in the first period to 67.7% (315/465) in the last (p<0.001).

For Gram-positives, penicillin resistance (including intermediate susceptibility) in *S. pneumoniae* did not increase significantly during the study period (p=0.210; Table 2). MRSA isolates increased from 0% (0/247) to 2.7% (6/224) which was not significant (p=0.09). *Enterococcus* spp. resistance to ampicillin increased from 11.1% (1/9) in the first period to 69.7% (69/99) in the last (p<0.001), which could be attributed to the seven-fold increase in *E. faecium* spp. isolated, concurrent with a relative decrease in *E. faecalis* over the same period.

For Gram-negatives, excluding Salmonellae, an increase in resistance to ceftriaxone and gentamicin to over 60% during the study period was documented (Figure 3A). *E. coli* ceftriaxone resistance increased from 11.1% (4/36) in the first period to 28.5% (47/165) in the last (p<0.001), gentamicin resistance increased from 19.7% (48/244) in the first period to 32.7% (54/165) in the last (p<0.001), and ampicillin resistance remained unchanged and had a mean annual rate of 89.7% throughout the study period (p=0.466). For *Klebsiella* spp., ceftriaxone resistance rose from 21.1% (4/19) in the first period to 90.5% (286/316) in the last (overall increasing trend, p<0.001), whereas gentamicin resistance rose from 38.2% (68/178) in the first period to 90.7% (282/311) in the last (overall increasing trend, p<0.001). Resistance to ciprofloxacin for both pathogens was also observed to steadily increase during the two decades, from 0% (0/113) in the first period to 26.0% (43/165) in the last (p<0.001) in *E. coli*, and 0% (0/73) to 34.5% (109/316) for *Klebsiella* spp (p<0.001). Resistance to all empiric first-line antimicrobials for *E. coli* was over 20% (Figure 3B) and there was an overall increasing trend in proportion of *Klebsiella* spp. resistant to all first-line antimicrobial regimens over time, from 5.9% (1/17) to 93.7% (133/142; p<0.001). Both *S.* Typhi and NTS had infrequent resistance to ceftriaxone (0/566 and 8/1,980) and ciprofloxacin (1/610 and

10/3,392) throughout all periods. We have not detected ESBL NTS BSI in children [18]. A similar trend in AMR profiles was also noted for young infants (Supplementary Table 3).

Discussion

Over the past two decades, overall, the incidence of BSI in hospitalized children at QECH, the largest teaching hospital in Malawi, has decreased. This includes NTS for which no vaccine was available during the study period, and for *H. influenzae* type b and *S. pneumoniae* for which vaccination programs were introduced (in 2002 and 2011, respectively). However, in the last ten years, the incidence of young infant BSI caused by *S.* Typhi and *Klebsiella* spp. in those under 5 has risen considerably. We have previously reported three epidemics of *Salmonella* which appear to have arisen through the acquisition of virulence and antimicrobial resistance determinants in the context of a susceptible population [19-22]. Gram-positive pathogens are still largely susceptible to first-line antimicrobials, with the exception of *Enterococcus* spp. and *E. faecium*, which, as an emerging pathogen, needs ongoing surveillance. For Gram-negative BSI among children ≤5 years, the proportion of bacteria that are resistant to empiric first-line antimicrobials is high, rising, and most marked among young infants. These findings reveal the growing problem of AMR in our setting and emphasize the need for robust antimicrobial stewardship programs and ongoing surveillance even when BSI overall appears to be declining.

The rise in Gram-negative pathogens resistant to first-line antimicrobials, specifically *Klebsiella* spp., contrasts with earlier data from our institution where isolates had high susceptibility (78%) [23]. One reason for the rise in AMR could be attributed to a marked increase in the use of broad-spectrum antimicrobials in recent years [24] including among neonates. *Klebsiella* spp. isolates recovered within the first 3 days of birth accounted for 28.4% (84/295) of all pediatric *Klebsiella* spp. specimens in the last period, suggesting high rates of vertical transmission. The reasons for increase in rates of AMR *Klebsiella*

spp. are not certain, since there have been no changes in practice in the neonatal unit that we can identify. The increase may have been amplified by increased transmission capacity resulting in outbreaks in neonatal units [25, 26]. The high ESBL carriage in children <5 years [27] may have also contributed to horizontal transmission. Community-based surveillance of both disease and carriage is ongoing [28] to explore whether increasing rates of AMR may be related to strengthening of primary health care systems following the model of IMCI and earlier treatment with antimicrobials, and thereby limited to the hospital setting.

Alternative antimicrobials for Gram-negatives in these settings could include use of amikacin, where resistance rates are much lower than gentamicin (6.3% compared to 66.0%, respectively); or ciprofloxacin, where resistance rates are already rising, and when used in children has to be weighed against the side effects of tendinopathy. The inclusion of meropenem on the Malawi national formulary in 2015 and its availability in hospitals may be beneficial in the short term but increased use is likely to drive further AMR, similar to the increase in ESBL organisms seen following ceftriaxone use. This would be in a setting where there are limited diagnostic facilities and where antimicrobial stewardship initiatives are beset by scarce resources.

Limitations to this study include that these are urban tertiary referral hospital-based data from a single site, and not national surveillance. However, such large-scale long-term routine surveillance data is rarely available in this setting at a national level. Hospital-based surveillance could have enriched for AMR, as susceptible BSI may have responded to empiric oral antibiotics in the community, and only the sickest children present to hospital. However, given the likely clinical severity of BSI, except for children with typhoid fever, and the absence of other pediatric inpatient facilities, this bias is unlikely to be substantial. We could not reliably differentiate community- from hospital-acquired bloodstream infections, which

could be particularly relevant to young infant BSI, where some neonates have prolonged stays and have follow-up blood cultures obtained prior to changes in antimicrobial regimens for presumed sepsis. Nosocomial BSI may have contributed to our relatively high recovery rate of 11.9% but many of the prominent pathogens are uncommonly associated with nosocomial transmission. As blood cultures are not routinely repeated if bacteria considered contaminants are isolated, we may have underestimated true pathogens; however, typical risk factors such as indwelling vascular devices or febrile neutropenia are extremely rare in this population and prematurity would not have accounted for a substantial proportion. ESBL screening was not introduced until 2003, and therefore ESBL-producing pathogens may have been circulating yet undetected before then. Changes made to breakpoints in subsequent BSAC versions would have led to wider use of 'intermediate' categories which, in our study, may have increased the number of Gram negatives regarded as resistant. In all pediatric BSI surveillance studies blood volumes for culture remain a challenge and therefore underestimates of pathogens such as *S. pneumoniae* and *S.* Typhi, particularly in infants are possible. Comprehensive clinical data was not available to evaluate the contribution of AMR to mortality, however, we have previously reported the mortality associated with specific pathogens [29-32].

Our results raise significant concerns about the growing issue of AMR, and highlight the urgent need to review empiric antimicrobial regimens, implement and enforce infection control practices, and undertake pragmatic trials of antimicrobial stewardship. However, in many LMICs, without local data, it remains challenging to adapt the WHO IMCI guidelines to reflect local needs [5]. While awaiting the impact of international initiatives to expand access to high quality laboratory surveillance [33, 34] and the development of rapid diagnostics [35, 36], there should be considerable emphasis on expanded access to effective antimicrobials in the context of locally relevant antimicrobial stewardship programs. These should include the education of community and hospital-based healthcare providers on prescribing practices, improving the understanding of epidemiology of infections, when not to prescribe antibiotics,

the development of and adherence to guidelines on de-escalation and cessation of antimicrobial therapy, and greater emphasis on infection control practices. The development of pragmatic clinical trials to evaluate new antimicrobial strategies in young children should be a priority.

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Conflict of interest

Dr. French reports grants from GSk, outside the submitted work. Dr. Tam reports grants from Bill and Melinda Gates Foundation, outside the submitted work. All other authors have no potential conflicts of interest.

References

1. UNICEF. One is too many: Ending child deaths from pneumonia and diarrhoea, **2016**.

2. Swann O, Everett DB, Furyk JS, et al. Bacterial meningitis in Malawian infants <2 months of age: etiology and susceptibility to World Health Organization first-line antibiotics. The Pediatric infectious disease journal **2014**; 33:560-5.

3. Molyneux EM, Dube Q, Banda F, et al. The Treatment of Possible Severe Infection in Infants: An Open Randomized Safety Trial of Parenteral Benzylpenicillin and Gentamicin Versus Ceftriaxone in Infants <60 days of Age in Malawi. The Pediatric infectious disease journal **2017**.

4. WHO. Integrated Management of Childhood Illness Chart Booklet. In: WHO, ed, **2014**.

5. Fleischmann-Struzek C, Goldfarb DM, Schlattmann P, Schlapbach LJ, Reinhart K, Kissoon N. The global burden of paediatric and neonatal sepsis: a systematic review. Lancet Respir Med **2018**; 6:223-30.

6. Daza P, Banda R, Misoya K, et al. The impact of routine infant immunization with Haemophilus influenzae type b conjugate vaccine in Malawi, a country with high human immunodeficiency virus prevalence. Vaccine **2006**; 24:6232-9.

7. Government of Malawi Ministry of Health. Integrated HIV Program Report July-September 2016. Malawi, **2016**.

8. WHO. Malawi Maternal and Child Health Data. Geneva, Switzerland: WHO, **2015**.

9. Jahn A, Floyd S, Crampin AC, et al. Declining child mortality in northern Malawi despite high rates of infection with HIV. Bull World Health Organ **2010**; 88:746-53.

10. Mwagomba B, Zachariah R, Massaquoi M, et al. Mortality reduction associated with HIV/AIDS care and antiretroviral treatment in rural Malawi: evidence from registers, coffin sales and funerals. PloS one **2010**; 5:e10452.

11. Zimba E, Kinney MV, Kachale F, et al. Newborn survival in Malawi: a decade of change and future implications. Health Policy Plan **2012**; 27 Suppl 3:iii88-103.

12. 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. Lancet Infect Dis **2017**; 17:1042-52.

13. WHO. Antimicrobial resistance: global report on surveillance. France, **2014**.

14. Senn N, Rarau P, Salib M, et al. Use of antibiotics within the IMCI guidelines in outpatient settings in Papua New Guinean children: an observational and effectiveness study. PLoS ONE [Electronic Resource] **2014**; 9:e90990.

15. Zurovac D, Rowe AK. Quality of treatment for febrile illness among children at outpatient facilities in sub-Saharan Africa. Annals of tropical medicine and parasitology **2006**; 100:283-96.

16. 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. Chest **1992**; 101:1644-55.

17. Gordon MA, Walsh AL, Chaponda M, et al. Bacteraemia and mortality among adult medical admissions in Malawi--predominance of non-typhi salmonellae and Streptococcus pneumoniae. J Infect **2001**; 42:44-9.

18. Feasey NA, Cain AK, Msefula CL, et al. Drug resistance in Salmonella enterica ser. Typhimurium bloodstream infection, Malawi. Emerging infectious diseases **2014**; 20:1957-9.

19. Feasey NA, Gaskell K, Wong V, et al. Rapid emergence of multidrug resistant, H58-lineage Salmonella typhi in Blantyre, Malawi. PLoS Negl Trop Dis **2015**; 9:e0003748.

20. Feasey NA, Hadfield J, Keddy KH, et al. Distinct Salmonella Enteritidis lineages associated with enterocolitis in high-income settings and invasive disease in low-income settings. Nat Genet **2016**; 48:1211-7.

21. Gordon MA, Graham SM, Walsh AL, et al. Epidemics of invasive Salmonella enterica serovar enteritidis and S. enterica Serovar typhimurium infection associated with multidrug resistance among adults and children in Malawi. Clinical infectious diseases **2008**; 46:963-9.

22. Kingsley RA, Msefula CL, Thomson NR, et al. Epidemic multiple drug resistant Salmonella Typhimurium causing invasive disease in sub-Saharan Africa have a distinct genotype. Genome Res **2009**; 19:2279-87.

23. Milledge J, Calis JC, Graham SM, et al. Aetiology of neonatal sepsis in Blantyre, Malawi: 1996-2001. Annals of tropical paediatrics **2005**; 25:101-10.

24. Feasey NA, Houston A, Mukaka M, et al. A reduction in adult blood stream infection and case fatality at a large African hospital following antiretroviral therapy roll-out. PloS one **2014**; 9:e92226.

25. Gurieva T, Dautzenberg MJD, Gniadkowski M, Derde LPG, Bonten MJM, Bootsma MCJ. The Transmissibility of Antibiotic-Resistant Enterobacteriaceae in Intensive Care Units. Clinical infectious diseases **2018**; 66:489-93.

26. Hilty M, Betsch BY, Bogli-Stuber K, et al. Transmission dynamics of extended-spectrum beta-lactamase-producing Enterobacteriaceae in the tertiary care hospital and the household setting. Clinical infectious diseases **2012**; 55:967-75.

27. Schaumburg F, Alabi A, Kokou C, et al. High burden of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Gabon. The Journal of antimicrobial chemotherapy **2013**; 68:2140-3.

28. Darton TC, Meiring JE, Tonks S, et al. The STRATAA study protocol: a programme to assess the burden of enteric fever in Bangladesh, Malawi and Nepal using prospective population census, passive surveillance, serological studies and healthcare utilisation surveys. BMJ Open **2017**; 7:e016283.

29. Fox-Lewis A, Takata J, Miliya T, et al. Antimicrobial Resistance in Invasive Bacterial Infections in Hospitalized Children, Cambodia, 2007-2016. Emerging infectious diseases **2018**; 24:841-51.

30. Kayange N, Kamugisha E, Mwizamholya DL, Jeremiah S, Mshana SE. Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza-Tanzania. BMC pediatrics **2010**; 10:39.

31. Laxminarayan R, Bhutta ZA. Antimicrobial resistance-a threat to neonate survival. Lancet Glob Health **2016**; 4:e676-7.

32. Thaver D, Ali SA, Zaidi AK. Antimicrobial resistance among neonatal pathogens in developing countries. The Pediatric infectious disease journal **2009**; 28:S19-21.

33. WHO. Guide for establishing laboratory-based surveillance for antimicrobial resistance. Available at: http://apps.who.int/medicinedocs/documents/s20135en/s20135en.pdf. Accessed 30 April 2018.

34. World Bank. AFCC2/RI-East Africa Public Health Laboratory Networking Project. Available at: http://projects.worldbank.org/P111556/east-africa-public-health-laboratory-networking-project?lang=en. Accessed 30 April 2018.

35. Amukele T. Africa CDC: Establishing Integrated Surveillance and Laboratory Networks for Rapid Disease Detection and Response, Control, Prevention, and Clinical Care in Africa. Afr J Lab Med **2017**; 6:638.

36. Peeling RW, Mabey D. Point-of-care tests for diagnosing infections in the developing world. Clinical microbiology and infection **2010**; 16:1062-9.

Tables and Figures:

Table 1. Bloodstream infections in children ≤5 years at Queen Elizabeth Central Hospital, by isolate and period

Table 2. Antimicrobial resistance profiles of selected bloodstream pathogens for children ≤5 years, by period

Figure legend

Figure 1. Negative binomial regression model estimated annual incidence rate per 1,000 person-years for children ≤5 years, for: A) All pathogenic organisms among those ≤5 years and ≤60 days; B) *S. pneumoniae* and *S. aureus*; C) Group A Strep and Group B Strep; D) *S.* Typhi and NTS; E) *E. coli* and *Klebsiella* spp.; F) *Haemophilus* spp. and other Gram-negatives. Scales have been adjusted for each organism.

Figure 2. Proportion of culture-confirmed bloodstream pathogens resistant to empiric first-line antimicrobials* by period, for children ≤5 years and ≤60 days

*First-line antimicrobials in Malawi are ampicillin/penicillin with gentamicin, or ceftriaxone.

Figure 3. Proportion of non-Salmonella Gram-negative isolates with: A) Gentamicin- or ceftriaxone-resistance, for children ≤5 years; B) *E. coli* and *Klebsiella* spp. resistance to empiric first-line antimicrobials*, for children ≤5 years

*First-line antimicrobials in Malawi are ampicillin/penicillin with gentamicin, or ceftriaxone.

Table 1. Bloodstream infections in children ≤5 years at Queen Elizabeth Central Hospital, by isolate and period

	Time period							
	1998-2002		2003-2007		2008-2012		2013-2017	
	N	%	N	%	N	%	N	%
Gram-positives								
Group A Strep	96	2.4	42	1.1	32	1.7	29	1.1
Group B Strep	126	3.2	116	2.9	39	2.0	48	1.8
Streptococcus pneumoniae	405	10.2	423	10.7	166	8.6	49	1.9
Staphylococcus aureus	247	6.2	283	7.1	185	9.6	253	9.7
Other *Streptococcus* spp.	197	5.0	244	6.1	193	10.0	222	8.5
E. faecalis	26	0.7	63	1.6	38	2.0	30	1.1
E. faecium	0	0	0	0	10	0.5	69	2.6
All *Enterococcus* spp.	31	0.8	63	1.6	50	2.6	99	3.8
Leuconostoc	0	0	1	0.03	0	0	0	0
Gram-negatives								
Acinetobacter baumanii	0	0	9	0.2	17	0.9	58	2.2
All *Acinetobacter* spp.	86	2.2	101	2.5	35	1.8	59	2.3

Citrobacter spp.	37	0.9	33	0.8	6	0.3	7	0.3
Enterobacter spp.	57	1.4	133	3.4	33	1.7	132	5.0
Escherichia coli	247	6.2	282	7.1	163	8.5	165	6.3
Haemophilus influenzae type b	157	4.0	44	1.1	19	1.0	16	0.6
All *Haemophilus* spp.	178	4.5	67	1.7	38	2.0	26	1.0
Klebsiella pneumoniae	57	1.4	103	2.6	123	6.4	295	11.3
All *Klebsiella* spp.	179	4.5	144	3.6	132	6.9	316	12.1
Neisseria meningitidis	14	0.3	18	0.5	5	0.3	11	0.4
N. gonorrhoea	0	0	0	0	1	0.05	6	0.3
Proteus spp.	15	0.4	3	0.08	2	0.1	3	0.1
Pseudomonas aeruginosa	28	0.7	47	1.2	20	1.0	36	1.4
All *Pseudomonas* spp.	38	1.0	55	1.4	33	1.7	49	1.9
Salmonella Typhi	8	0.2	16	0.4	50	2.6	544	20.8
NTS	1644	41.4	1505	37.9	532	27.7	303	11.6
Serratia spp.	38	1.0	16	0.4	4	0.2	17	0.7
Shigella spp.	3	0.08	4	0.1	4	0.2	6	0.2
Vibrio spp.	0	0	0	0	2	0.1	0	0
Yersinia spp.	2	0.05	0	0	2	0.1	0	0
Other Gram-negatives[a]	301	7.6	412	10.4	203	10.6	253	9.7

Fungus								
Candida	5	0.1	1	0.03	4	0.2	7	0.3
Cryptococcus	0	0	0	0	6	0.3	4	0.2
Yeast species	0	0	0	0	0	0	3	0.1
All pathogens[b]	3964	100	3970	100	1923	100	2614	100

MRSA, methicillin-resistant Staph aureus; NTS, nontyphoidal Salmonella

[a]Includes *Aeromonas* spp., *Agrobacter* spp., *Burkholderia* spp., *Cronobacter* spp., *Edwardsiella* spp., Flavobacteria, Gram negative rods, *Hafnia* spp., *Histophilus* spp., *Kluyvera* spp., *Moraxella* spp., *Morganella* spp., *Pantoea* spp., *Pasteurella* spp., *Raoultella* spp., *Sphingomonas* spp., *Stenotrophomonas* spp., *Xanthomonas* spp..

[b]Excludes contaminants, including *Aerococcus* spp., alpha-hemolytic streptococci, *Alcaligenes* spp., *Bacillus* spp., *Clostridium* spp., coagulase-negative staphylococci, Corynebacteria, Diphtheroids, Gram positive rods, *Rhizobium* spp., *Micrococcus* spp., skin flora.

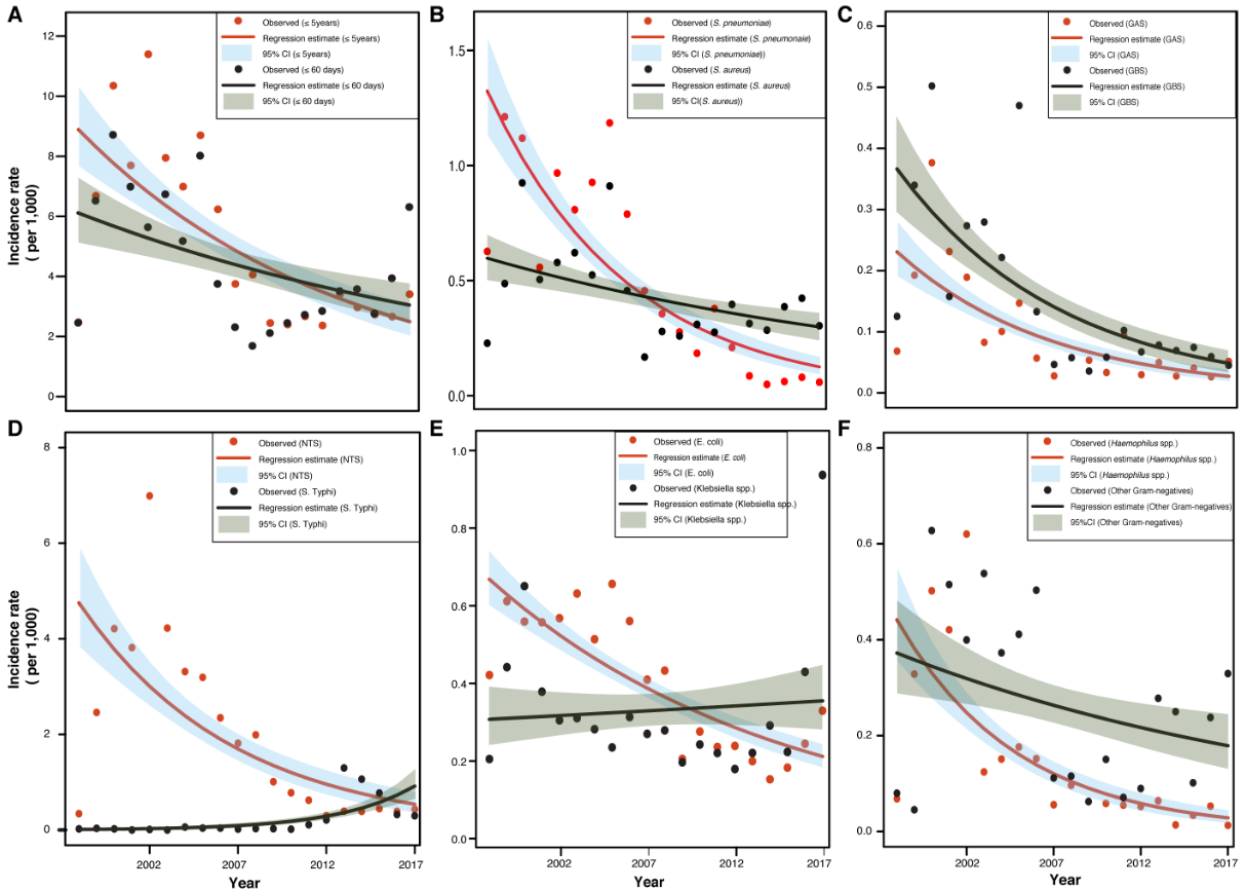

Figure 1

Figure 2

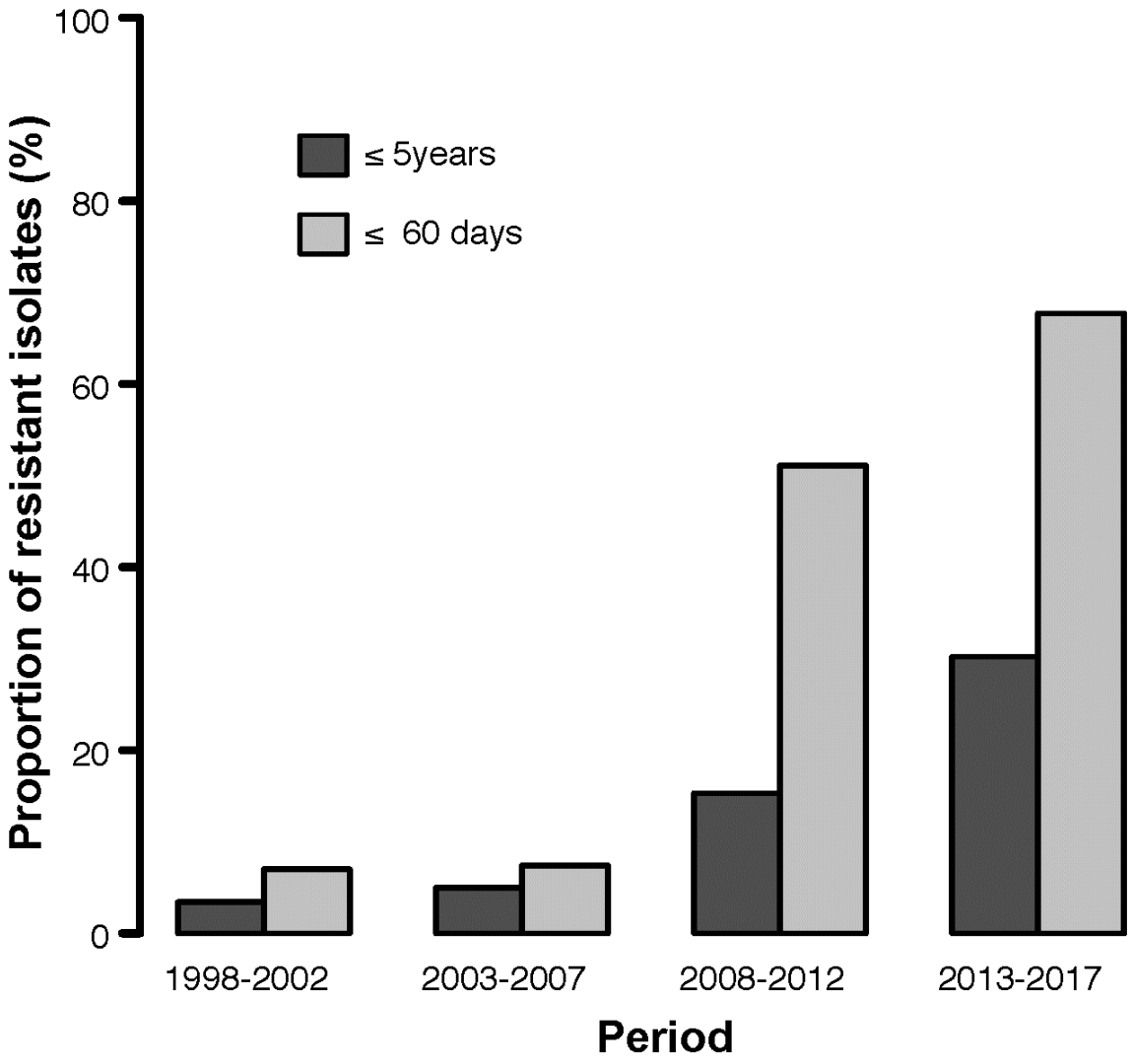

Figure 3