Prevalence of Extended-Spectrum β -Lactamase Producing *Enterobacteriaceae* in Community Patients in Blantyre, Malawi

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Research Article

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Abstract

Background

Antimicrobial resistance due to production of extended-spectrum β -lactamase (ESBL) by *Enterobacteriaceae* is a global health problem contributing to increased morbidity and mortality particularly in resource-constrained countries. We examined in the current study the prevalence of extended spectrum β -lactamase producing *Enterobacteriaceae* (ESBL-E) in community patients in Blantyre, Malawi.

Methods

This was a cross sectional study conducted between March and September 2020 at selected outpatient health facilities in Blantyre, Malawi. Clinical samples were first screened for ESBL-E using CHROMagar™ ESBL medium and later confirmed by a combination disk test method (CDT). The isolates were identified to species level using a commercially acquired biochemical substrate strips (Microbact™ GNB, Oxoid). Descriptive summary statistics were generated as frequencies and proportions. Chi square or Fishers Exact tests and student's *t*-test were used where appropriate. Association between variables was determined by logistic regression analysis. Results were presented as odds ratio and 95% confidence interval. A *p*-value ≤ 0.05 was regarded as statistically significant.

Results

A total of 199 rectal swabs and 101 urine samples from 300 outpatient adults were screened for ESBL-E. Of these, seventy three (24.33%; 95% CI = 19.45–29.22%) gave positive culture. Prevalence of community acquired ESBL-E was 16.67% (50/300, 95% CI = 12.43–20.91%). The most common ESBL-E species isolated were *Escherichia coli* (66%). Community prevalence of ESBL-E was higher in male 56% (28/50) compared to female 44% (22/50) patients. Prevalence of ESBL-E was higher in community patients who lacked history of surgery 88% (44/50) and prior history of antibiotic use in the last three months 80% (40/50) compared to those with same characteristics. All community patients with isolated ESBL-E had no history of admission in the last three months and neither demographic characteristics nor clinical characteristics of participants showed any degree of association with the carriage of ESBL-E.

Conclusion

Our findings revealed moderate presence of ESBL-E phenotypes in a community patients who lacked history of hospital admissions in the past three months an indication of community acquisition of ESBL-E in Blantyre, Malawi. Low prevalence of ESBL-E in the community settings in Blantyre can be maintained if strong infection and antimicrobial use control strategies are to be implemented.

Introduction

Extended-spectrum β -lactamase producing *Enterobacteriaceae* (ESBL-E) were exclusively observed in hospital settings in early years after the introduction of Cephalosporins in 1990s [1, 2]. They continue to be a major global health challenge in both clinical and infection prevention control. Currently global problem of antimicrobial resistance (AMR) resulting from the production of ESBL enzymes by *Enterobacteriaceae* has increased to an alarming magnitude and is among the leading threats to human health [3]; the most affected are the developing countries with limited resources to implement control measures to curb AMR [4]. Increased spread of ESBL-E is not only occurring in hospital settings but also in the communities. The prevalence of community acquired ESBL-E worldwide remained below 10% before 2008 but has rapidly exploded to over 60% in recent years causing a major challenge for antibiotic therapy to both nosocomial and community acquired infections [5, 6]

In low income countries, lack of enforcement of regulations and guidelines controlling the sale and irrational use of antibiotics in both animal and humans contributed to the emergence and widespread of resistant bacteria [7]. The problem of AMR as a result of failure to treat bacteria that are resistant to locally available antimicrobial agents is implicated in the longer hospitalization and the choice for more expensive drugs which are often not readily available in the resource constrained countries like Malawi [8, 9].

Few available studies conducted in Malawi to account for the presence of ESBL producing *Enterobacteriaceae* in humans were based on observational study and surveillance data from hospital settings [10–12]. In 2005, prevalence of ESBL producing *Enterobacteriaceae* reported after baseline review of ESBL problem and characterization of ESBL enzymes from clinical isolates following the death of patients attributed to multidrug resistant *Klebsiella pneumoniae* infection was found to be 0.7% [11]. Twelve years later (2017), the study describing longitudinal trends in AMR at a teaching hospital in Blantyre Malawi that focused on prevalence of ESBL resistance among Gram-negative bacteria causing blood stream infections found that the magnitude of ESBL producing *Enterobacteriaceae* rose in *Escherichia coli* from 0.7 to 30.3%, 11.8 to 90.5% in *Klebsiella* spp and 30.4 to 71.9% in other *Enterobacteriaceae* [12].

However, the current status of ESBL producing *Enterobacteriaceae* burden in the community settings in Malawi and Blantyre in particular is lacking. As an evidence of the level of ESBL producing *Enterobacteriaceae* carriage in the communities in Blantyre, ESBL producing *E. coli* has been recovered from a stool of a patient with neither history of admission nor travel history in six months [13]. Therefore, the current study was conducted to provide baseline prevalence and associated factors for carriage of ESBL β -lactamase producing *Enterobacteriaceae* in community patients in Blantyre, Malawi.

Material And Methods

Study design, duration and location

This was a cross-sectional study carried out between March and September 2020 at three randomly selected outpatient health centres representing community settings in Blantyre, Malawi (Figure.1). Outpatient health centres were chosen as a point of data collection due to conveniences in sample collection and storage and these were Limbe, Zingwangwa and Ndirande health centres. These health centres are the main public health care facilities providing outpatient services to a population of over 800,264 of Blantyre district.

Study population, sample size and sampling

Study participants comprised of 300 outpatient adults from three main public health centres representing community settings in Blantyre. Sample size was calculated using a formula described by Lwanga and Lemeshow [14] at 95% confidence interval based on 61.9% prevalence of ESBL producing *Enterobacteriaceae* in Blantyre as previously reported [12]. All adult patients (≥ 18 years old) who presented on the day of data collection had equal chances to be included in the study. Patients who were willing and consented to take part in the study were randomly recruited in the study regardless of their reason to seek medical care at the health centres.

Specimen collection

Clinical specimens (urine and rectal swabs) were collected and handled following standard protocol by registered and experienced health personnel working at each health centre in accordance [to ethical principles for medical research involving human subjects](#). Only one type of clinical specimen was collected from each participant. Urine samples were collected exclusively from outpatients who presented with UTI related complains using sterile urine-cup and rectal swabs were collected using Amies flocced swabs (collection and transport system) (COPAN, Brescia-Italia). Collected urine and swabs were transported to Microbiology Laboratory, College of medicine, University of Malawi for culture of ESBL producing *Enterobacteriaceae*.

Culture of ESBL producing *Enterobacteriaceae*

Culture of ESBL producing *Enterobacteriaceae* was performed on CHROMagar™ ESBL medium supplemented with ESBL supplement containing a selective mixture of antibiotics enabling selective growth of ESBL producing *Enterobacteriaceae* and inhibiting growth of non-ESBL *Enterobacteriaceae* (CHROMagar, Paris, France). Samples were processed by direct inoculation onto CHROMagar™ ESBL plates using streaking and spreading techniques followed by cover bottom side incubation in aerobic conditions at 37°C for 18-24 hours. Following incubation, a significant growth of ESBL producing *Enterobacteriaceae* and appearance of the colonies were observed.

Phenotypic confirmation of ESBL production by *Enterobacteriaceae*

Phenotypic confirmation of ESBL production by the isolates was done using a combination disk test method (CDT) by comparing inhibition zone diameter around a cephalosporin disks to that of the same

cephalosporin plus clavulanate following the recommendations of Clinical and Laboratory Standards Institute (CLSI) 2017. In the current study, we used MAST combination disks (MAST D52C ESBL, Mast Diagnostics, Merseyside, UK) to phenotypically confirm ESBL production in *Enterobacteriaceae* isolates with no chromosomal de-repressed or inducible AmpC that grew on CHROMagar™ ESBL medium. Both cefotaxime ((CTX-30µg) and ceftazidime (CAZ-30µg) antibiotic disks with and without clavulanic acid (CA-10µg) were used concurrently based on comparing the zones of cefotaxime and cefotaxime plus clavulanate and ceftazidime disk with and without clavulanic acid. The increase in zone diameter of ≥ 5 mm or a zone expansion of 50% i.e. corresponding to a two-fold dilution between the zone of inhibition of a single disk and in combination with clavulanic acid was indicative of ESBL production as previously described [15].

Quality control

ESBL producing *Klebsiella pneumonia* (ATCC 700603) and non-ESBL producing *E. coli* (ATCC 25922) were used as positive and negative control, respectively.

Identification of *Enterobacteriaceae* species

Presumptive identification of common ESBL producing *Enterobacteriaceae* isolates was done based on colony color characteristics of bacteria growth on CHROMagar™ plates according to manufacturer's instructions; i.e. ESBL producing *Escherichia coli* was identified by dark pink color, ESBL producing KEC (*Klebsiella*, *Enterobacter*, *Citrobacter*) by metallic blue +/-reddish halo), ESBL producing *Proteus* by brown halo, ESBL producing *Acinetobacter* by cream color and ESBL producing *Pseudomonas* was translucent (+/- natural).

The identity of isolates were subsequently confirmed using the commercially acquired biochemical substrate strips (Microbact™ gram negative identification system, Oxoid, GNB 12A). The standardized mirco-substrate strips (Microbact™) were inoculated according to the manufacturer's instructions for identification of *Enterobacteriaceae*. The biochemical tests used were Lysine, Ornithine, H₂S, Glucose, Mannitol, Xylose, ONPG, Indole, Urease, VP, Citrate and TDA. The interpretation to identify the isolates was done using the Microbact™ computer aided identification package (Oxoid) in combination with the Cowan and Steel's Manual for the Identification of Medical Bacteria [16]

Statistical analysis

Data obtained were cleaned and transferred to STATA version 12.0 (Stata Corp LP, College Station, USA) for statistical analysis. Descriptive summary statistics were generated as frequencies and proportions presented in tabular form. Chi square and Fishers Exact tests were used to compare dichotomous variables as appropriate. Univariate association between ESBL-E positivity (outcome) and independent variables was determined by logistic regression analysis. When fitting the model, participants who had separated, divorced, widow and single marital status were combined to obtain single variable (unmarried)

and was compared with married or cohabiting participants. Results were presented as odds ratio and 95% confidence interval. A p-value ≤ 0.05 was regarded as statistically significant.

Results

Social demographic and clinical characteristics of the study population

A total of 300 adult patients attending outpatient clinics in Blantyre district were enrolled in the study. The median age of participants was 29.5 years (IQR=23-38; range, 18-75 years). Participant's male to female sex ratio was 1:1.2. Majority of participants (41.67%) were between the ages of 18-27 years, married or cohabiting (61.33%), had primary education (44.33%) and were unemployed (46%). In the past three months, more than ninety seven percent of participants had no history of admission, 90.33% had no history of surgery and 78% had not used antibiotics (Table 1).

Table 1: Characteristics of study population by ESBL-E phenotype

Variables	Frequency (%)	ESBL phenotype		p-value
		Positive n(%)	Negative n(%)	
Age				0.63
18-27	125(41.67%)	20(6.67%)	105(35.00%)	
28-37	95(31.67%)	13(4.34%)	82 (27.33%)	
38-47	43(14.33%)	10(3.33%)	33 (11.00%)	
48-57	20(6.67%)	3(1.00%)	17(5.67%)	
≥58	17(5.67%)	4(1.33%)	13(4.34%)	
Sex				0.11
Male	137(45.67%)	28(9.33%)	109 (36.33%)	
Female	163(54.33%)	22 (7.34%)	141(47.00%)	
Marital status				0.05
Separated, divorced or widow	23(7.67%)	8(2.67%)	15(5.00%)	
Single(never married)	93 (31.00%)	14 (4.67%)	79 (26.33%)	
Married or cohabiting	184 (61.33%)	28 (9.33%)	156 (52.00%)	
Education				0.54
Primary	133 (44.33)	23 (7.67%)	110 (36.67%)	
Secondary	115 (38.33%)	16 (5.33%)	99 (33.00%)	
College/University	6 (2.00%)	2 (0.67%)	4 (1.33%)	
Didn't attend any school	46 (15.33%)	9 (3.00%)	37(12.33%)	
Occupation				0.29
Unemployed	138(46%)	24(8.00%)	114(38.005)	
Self-employment or business	57(19%)	12(4.00%)	45(15.00%)	
Employed	78(26%)	8(2.67%)	70(23.33%)	
Student	27(9%)	6(2.00%)	21(7.00%)	
History of prior antibiotic use in last 3 months				0.71
Yes	66 (22.00%)	10 (3.33%)	56 (18.67%)	
No	234 (78.00%)	40 (13.33)	194 (64.67%)	

History of surgery in previous 3 moths			0.54
Yes	29 (9.67%)	6 (2.00%)	23 (7.67%)
No	271 (90.33%)	44(14.67)	227 (75.66%)
History of admission in previous 3 moths			0.23
Yes	7 (2.33%)	0(0.00%)	7(2.33%)
No	293 (97.67%)	50 (16.67%)	243(81.00)
Outpatient health centre			0.20
Limbe	99(33.00%)	13(4.33%)	86(28.67)
Ndirande	100(33.33%)	22(7.33%)	78(26.00%)
Zingwangwa	101(33.67)	15(5.00%)	86(28.67)

Prevalence of ESBL producing *Enterobacteriaceae* in community patients in Blantyre

Of the 300 samples screened for potential ESBL *Enterobacteriaceae* phenotypes, 73/300 (24.33%; 95%, CI=19.45-29.22%) gave positive culture; 20% from rectal swabs and 4.33% from urine. The overall rate of ESBL-E carriage in community patients was 16.67% (50/300, 95% CI=12.43-20.91%), 14% rectal and 2.67% in urine.

Of the 50 ESBL producing *Enterobacteriaceae* isolates recovered from outpatient adults in Blantyre, 84% were from rectal swabs. The most abundant ESBL-E species isolated were *Escherichia coli* (66%) followed by *Klebsiella spp* (8%), *Yersinia enterocolitica* (6%), *Enterobacter lwofii* (4%) and others (Table 2). Community prevalence of ESBL-E was higher in male 56% (28/50) compared to female 44% (22/50); however, this difference was not statistically significant $X^2=2.5821$, $p=0.11$. Prevalence of ESBL-E was higher in married or cohabiting 56% (28/50), unemployed 48% (24/50) and those with primary education 46% (23/50). Community patients who lacked history of surgery and history of antibiotic use in the past three months 88% (44/50) and 80% (40/50) respectively had high rate of ESBL producing *Enterobacteriaceae* carriage compared to those who underwent surgery 12% (6/50) and who used antibiotics in previous three months 20% (10/50). We found that all community patients with ESBL-E carriage had no history of admission in the last three months (Table 1).

Table 2: Composition of ESBL producing *Enterobacteriaceae* isolates from clinical samples (N=50)

ESBL <i>Enterobacteriaceae</i>	Rectal swab	Urine	Total n (%)
<i>Acinetobacter baumannii</i>	1	0	1 (2%)
<i>Acinetobacter Iwoffii</i>	2	0	2 (4%)
<i>Enterobacter Aerogens</i>	1	0	1 (2%)
<i>Enterobacter agglomerans</i>	1	1	2 (4%)
<i>Escherichia Coli</i>	29	4	33 (66%)
<i>Klebsiella Oxytoca</i>	2	0	2 (4%)
<i>Klebsiella pneumoniae</i>	2	0	2 (4%)
<i>Providencia rettgeri</i>	0	1	1 (2%)
<i>Serratia liquefaciens</i>	0	1	1 (2%)
<i>Serratia rubidaea</i>	0	1	1 (2%)
<i>Shigella sonnei</i>	1	0	1 (2%)
<i>Yersinia enterocolitica</i>	3	0	3 (6%)
Total	42 (84%)	8 (16%)	50(100%)

Univariate analysis

The summary of unadjusted logistic regression model performed to establish the relationship between patient characteristics and carriage of ESBL-E in community patients is presented in Table 3. Neither demographic characteristics nor clinical characteristics of participants showed any degree of association with the carriage of extended spectrum β -lactamase producing *Enterobacteriaceae*.

Table 3: Univariate logistic regression analysis of factors associated with carriage of ESBL producing *Enterobacteriaceae* in community patients.

Associated factor	Odds Ratio (95%, CI)	p-value
Age		
18-27	0.62(0.18-2.09)	0.44
28-37	0.52(0.15-1.82)	0.30
38-47	0.98(0.26-3.71)	0.98
48-57	0.57(0.12-3.02)	0.51
Sex		
Males	1.65 (0.89-3.04)	0.11
Marital status		
Married or cohabiting	0.77(0.41-1.42)	0.34
Education		
Primary	0.86 (0.36-2.02)	0.71
Secondary	0.66 (0.27-1.63)	0.37
College/University	2.06 (0.32-13.03)	0.45
Occupation		
Unemployed	0.72(0.12-1.28)	0.12
Self-employment or business	0.93(0.31-2.83)	0.90
Employed	0.4(0.12-1.28)	0.12
History of surgery in previous 3 months (Yes)		
	1.35 (0.52-3.49)	0.54
History of antibiotic use in last 3 months (Yes)		
	0.87(0.41-1.84)	0.71

Discussion

Despite the rise in extended spectrum β -lactamase producing *Enterobacteriaceae* prevalence worldwide, very few reports of community ESBL-E are available from Africa. Less commonly, detection of ESBL *Enterobacteriaceae* has been carried out from hospital settings and rarely from the community settings in developing countries including Malawi [10, 12, 17–19]. In this study, we demonstrated moderate prevalence and lack of factors associated with extended-spectrum β -lactamase producing *Enterobacteriaceae* in community settings in Blantyre, Malawi. Examining the status of ESBL producing *Enterobacteriaceae* burden and factors associated with contracting strains of ESBL-E in community

settings could provide important information that is a requisite for formulation and implementation of strong infection and antimicrobial use control strategies in both community and hospital settings.

Moderate prevalence found in the present study are similar to findings of low to moderate prevalence of ESBL-E in the community settings that were reported in Central Africa republic [20] and Kenya [21, 22]. Contrary to higher prevalence of ESBL-E that was previously reported in hospital setting in Blantyre [12], moderate prevalence observed in this study may be an indication that community acquired ESBL-E is still a minor problem in Blantyre, Malawi. Elsewhere it has been reported that higher rates of antibiotic consumption accelerate both AMR selection and higher rate of nosocomial ESBLs in hospital settings compared to community settings [23, 24], this can account for the moderate rate of ESBL-E detected in community patients in the current study.

We found a preponderance of ESBL-E among males compared to females, but there was no statistically significant difference between ESBL carriage in males and females. This finding is in conformity to findings of the study by Shah *et al.* [25] which reported ESBL positive isolates mostly in males (65.33%) compared to females (34.67%) when relating age and gender to extended-spectrum β -lactamases in *Enterobacteriaceae*. Another similar results were obtained in Bhopal region of Central India where 52.54% of ESBL isolates were obtained from males while 43.46% from females [26]. However, our findings differ from the findings of most studies conducted in hospital settings [12, 27–29]. It has been reported that urinary tract infections mostly affecting women and samples of urine are the common sources of bacteria isolates in most microbiology laboratories in different countries [30], whether sample type (rectal vs urine) has something to do with male/female differences in ESBL-E carriage is worth another study.

In the current study, all patients with ESBL-E carriage had no history of hospital admission and there was higher prevalence of ESBL-E in community patients who lacked history of surgery and history of antibiotic use in the past three months, these findings indicated that there was community acquisition of ESBL-E in Blantyre Malawi but at a moderate level.

Similar to studies that reported *E. coli* as the most frequent *Enterobacteriaceae* isolates in Ethiopia [31], Burkina Faso [32], Netherlands [33], Uganda [34] and India [26, 35], the current study showed that *Escherichia coli* were the most abundant *Enterobacteriaceae* species harboring extended-spectrum β -lactamase followed by *Klebsiella* spp in community patients in Blantyre. Probably, the reason for *E. coli* to contribute more than 60% of all isolates in the current study could be the community setting and the type of sample taken. Like our study in which rectal samples constituted greater number of clinical samples than urine, *E. coli* has mostly been isolated from faecal matter compared to urine[36]. Contrary to the current study where participants with ESBL-E carriage were adult community patients who did not report any history of hospital admission in previous three months, other *Enterobacteriaceae* species specifically *Klebsiella* spp have been found to cause a substantial morbidity among paediatric patients accounting for almost more than 50% of all Gram-negative infections in neonates and a significant burden of hospital-acquired infections in sub Saharan Africa [30].

It has been highlighted in the past that the risk factors for introduction of ESBL-E into the community includes travel to areas with a higher prevalence of ESBL pathogens, previous hospitalization, antibiotic treatments, old age, comorbidities like diabetes and previous infection by members of *Enterobacteriaceae* [37–39]. However, our study found that there was no relationship between ESBL-E carriage in community patients and their demographic or clinical characteristics similar to the study in The Gambia which found that most of the demographic characteristics had no strong association with the carriage of ESBL producing *Enterobacteriaceae* [40]. Lack of association between ESBL-E carriage and demographic or clinical characteristics of patients in this study may be due to the fact that all patients with ESBL-E carriage in the current study had no history of admission and few had history of prior antibiotic use in the past three months which are the main predictors of ESBL acquisition.

Limitations

In this study we used both cefotaxime and ceftazidime disks to confirm ESBLs and this was important because ESBLs could be missed if just a single disk was used. However our study could be limited by the accuracy and discriminatory power of the medium used during initial screening of ESBLs between bacteria producing these enzymes and those with other mechanisms of resistance to beta-lactams e.g., inhibitor-resistant β -lactamase and cephalosporinase overproduction. In addition to that, we were not able to perform genotypic characterization to determine the presence of ESBL genes in *Enterobacteriaceae* recovered from community patients.

Conclusion

Our findings revealed moderate presence of ESBL-E phenotypes in community patients, participants with ESBL-E carriage lacked history of hospital admission three months before the study. This can suggest community acquisition of ESBL-E in Blantyre, Malawi and should these carrier patients in the community remain untreated, they may serve as a community reservoir of resistant pathogens potential for the transmission and spread of community acquired ESBLs. Low prevalence of ESBL-E in community settings in Blantyre Malawi observed in this study can be maintained if strong infection and antimicrobial use control strategies are to be implemented.

Abbreviations

AMR	Antimicrobial resistance
ATCC	American type culture collection
CA	Clavulanic acid
CAZ	Ceftazidime
CDT	Combination disk test
CI	Confidence interval
CLSI	Clinical laboratory standards institute
COMREC	College of medicine research ethics committee
CTX	Cefotaxime
ESBL	Extended spectrum β -lactamase
ESBL-E	Extended spectrum β -lactamase producing <i>Enterobacteriaceae</i>
IQR	Interquartile range
KEC	<i>Klebsiella</i> , <i>Enterobacter</i> and <i>Citrobacter</i> spp
ONPG	O-Nitrophenyl- β -D-Galactopyranoside
TDA	Tryptophan deaminase
USA	United states of America

Declarations

Ethics approval and consent to participate

The study received ethical approval from the College of Medicine Research Ethics Committee (COMREC) of the University of Malawi (Certificate of Ethics Approval No. P.07/19/2720 of 22 November, 2019). Permission to conduct the study in health centres was obtained from Blantyre district health authority. Before enrolment into the study, a written informed consent was obtained from participants using a form translated in local language of Chichewa. For the patients without formal education attended, informed consent was obtained from legally authorized representatives.

Consent for publication

Not applicable

Availability of data and materials

The dataset used and/or analysed in the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare no competing interests

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Authors' contributions

OGO conceptualized, designed, collected and analyzed the data and drafted the manuscript. RSM, SA and SFR reviewed and contributed to content. All authors approved the final manuscript.

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References

1. Jacoby GA, Medeiros AA. More extended-spectrum β -lactamases. *Antimicrobial Agents and Chemotherapy* 1991; 35: 1697–1704.
2. 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.
3. Tacconelli E, Carrara E, Savoldi A, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* 2018; 18: 318–327.
4. Rossolini GM, Mantengoli E. Antimicrobial resistance in Europe and its potential impact on empirical therapy. In: *Clinical Microbiology and Infection*. Blackwell Publishing Ltd, pp. 2–8.
5. 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. *Clin Microbiol Rev* 2013; 26: 744–58.
6. Sasaki T, Hirai I, Niki M, et al. High prevalence of CTX-M β -lactamase-producing Enterobacteriaceae in stool specimens obtained from healthy individuals in Thailand. *J Antimicrob Chemother* 2010; 65: 666–668.
7. Ayukekbong JA, Ntemgwa M, Atabe AN. The threat of antimicrobial resistance in developing countries: Causes and control strategies. *Antimicrobial Resistance and Infection Control* 2017; 6: 47.

8. Friedman ND, Temkin E, Carmeli Y. The negative impact of antibiotic resistance. *Clinical Microbiology and Infection* 2016; 22: 416–422.
9. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: A global multifaceted phenomenon. *Pathogens and Global Health* 2015; 109: 309–318.
10. Musicha P, Msefula CL, Mather AE, et al. Genomic analysis of *Klebsiella pneumoniae* isolates from Malawi reveals acquisition of multiple ESBL determinants across diverse lineages. *J Antimicrob Chemother* 2019; 74: 1223–1232.
11. Gray KJ, Wilson LK, Phiri A, et al. Identification and characterization of ceftriaxone resistance and extended-spectrum β -lactamases in Malawian bacteraemic Enterobacteriaceae. *J Antimicrob Chemother* 2006; 57: 661–665.
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: 1042–1052.
13. Lewis JM, Lester R, Mphasa M, et al. Emergence of carbapenemase-producing Enterobacteriaceae in Malawi. *J Glob Antimicrob Resist* 2020; 20: 225–227.
14. Lwanga S, Lemeshow S. Sample size determination in health studies: A practical manual. *World Heal Organ Geneva* 1991; 88: 1–22.
15. M'Zali FH, Chanawong A, Kerr KG, et al. Detection of extended-spectrum β -lactamases in members of the family Enterobacteriaceae: comparison of the MAST DD test, the double disc and the Etest ESBL. *J Antimicrob Chemother* 2000; 45: 881–885.
16. Phillips I. Cowan and Steel's Manual for the Identification of Medical Bacteria. *J Clin Pathol* 1993; 46: 975.
17. 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: 422–429.
18. Moses A, Bwanga F, Boum Y, et al. Prevalence and Genotypic Characterization of Extended-Spectrum Beta-Lactamases Produced by Gram Negative Bacilli at a Tertiary Care Hospital in Rural South Western Uganda. *Br Microbiol Res J* 2014; 4: 1541.
19. Irengue 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: 1–6.
20. Janatova M, Albrechtova K, Petrzelkova KJ, et al. Antimicrobial-resistant Enterobacteriaceae from humans and wildlife in Dzanga-Sangha Protected Area, Central African Republic. *Vet Microbiol* 2014; 171: 422–431.
21. Kiiru J, Kariuki S, Goddeeris BM, et al. Analysis of β -lactamase phenotypes and carriage of selected β -lactamase genes among *Escherichia coli* strains obtained from Kenyan patients during an 18-year period. *BMC Microbiol* 2012; 12: 1471–2180.
22. Maina D, Revathi G, Kariuki S, et al. Genotypes and cephalosporin susceptibility in extended-spectrum beta-lactamase producing enterobacteriaceae in the community. *J Infect Dev Ctries* 2011;

6: 470–477.

23. Almagor J, Temkin E, Benenson I, et al. The impact of antibiotic use on transmission of resistant bacteria in hospitals: Insights from an agent-based model. *PLoS One*, 13. Epub ahead of print 1 May 2018. DOI: 10.1371/journal.pone.0197111.
24. Aslam B, Wang W, Arshad MI, et al. Antibiotic resistance: a rundown of a global crisis. *Infection and Drug Resistance* 2018; 11: 1645–1658.
25. Shah AA, Hasan F, Ahmed S, et al. Extended-spectrum β -lactamases in enterobacteriaceae: Related to age and gender. *New Microbiol* 2002; 25: 363–6.
26. Shashwati N, Kiran T, Dhanvijay A. Study of extended spectrum β -lactamase producing Enterobacteriaceae and antibiotic coresistance in a tertiary care teaching hospital. *J Nat Sci Biol Med* 2014; 5: 30.
27. Tellevik MG, Blomberg B, Kommedal Ø, et al. High Prevalence of Faecal Carriage of ESBL-Producing Enterobacteriaceae among Children in Dar es Salaam, Tanzania. *PLoS One* 2016; 11: e0168024.
28. 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.
29. 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: 1–6.
30. Williams PCM, Isaacs D, Berkley JA. Antimicrobial resistance among children in sub-Saharan Africa. *The Lancet Infectious Diseases* 2018; 18: e33–e44.
31. Teklu DS, Negeri AA, Legese MH, et al. Extended-spectrum beta-lactamase production and multi-drug resistance among Enterobacteriaceae isolated in Addis Ababa, Ethiopia. *Antimicrob Resist Infect Control* 2019; 8: 39.
32. Ouedraogo AS, Sanou M, Kissou A, et al. High prevalence of extended-spectrum β -lactamase producing enterobacteriaceae among clinical isolates in Burkina Faso. *BMC Infect Dis* 2016; 11: 326.
33. van den Bunt G, van Pelt W, Hidalgo L, et al. Prevalence, risk factors and genetic characterisation of extended-spectrum beta-lactamase and carbapenemase-producing Enterobacteriaceae (ESBL-E and CPE): a community-based cross-sectional study, the Netherlands, 2014 to 2016. *Eurosurveillance* 2019; 24: 1–11.
34. Najjuka CF, Kateete DP, Kajumbula HM, et al. Antimicrobial susceptibility profiles of Escherichia coli and Klebsiella pneumoniae isolated from outpatients in urban and rural districts of Uganda. *BMC Res Notes* 2016; 9: 235.
35. Rao SP, Rama PS, Gurushanthappa V, et al. Extended-spectrum beta-lactamases producing Escherichia coli and Klebsiella pneumoniaei: A Multi-centric Study Across Karnataka. *J Lab Physicians* 2014; 6: 007–013.
36. Mahamat OO, Lounnas M, Hide M, et al. High prevalence and characterization of extended-spectrum β -lactamase producing Enterobacteriaceae in Chadian hospitals. *BMC Infect Dis* 2019; 19: 1–7.

37. Paltansing S, Vlot JA, Kraakman MEM, et al. Extended-spectrum β -lactamase-producing enterobacteriaceae among travelers from the Netherlands. *Emerg Infect Dis* 2013; 19: 1206–1213.
38. Kantele A, Laaveri T, Mero S, et al. Antimicrobials Increase Travelers' Risk of Colonization by Extended-Spectrum Betalactamase-Producing Enterobacteriaceae. *Clin Infect Dis* 2015; 60: 837–846.
39. Peirano G, Laupland KB, Gregson DB, et al. Colonization of returning travelers with CTX-M-producing *Escherichia coli*. *J Travel Med* 2011; 18: 299–303.
40. Sanneh B, Kebbeh A, Jallow HS, et al. Prevalence and risk factors for faecal carriage of Extended Spectrum β -lactamase producing Enterobacteriaceae among food handlers in lower basic schools in West Coast Region of The Gambia. *PLoS One* 2018; 13: e0200894.

Figures

Figure 1

Map of Malawi (left) showing location of Blantyre district and map of Blantyre (right) showing the location of health centres Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.