Lack of Association Between Patients Characteristics and the Carriage of Extended-spectrum β-Lactamase Producing Enterobacteriaceae in Community Settings in Blantyre, Malawi

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Research note

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Abstract

Objective: This study examined factors associated with the carriage of extended-spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* in community patients in Blantyre, Malawi.

Results

A total of 50 community patients with ESBL producing *Enterobacteriaceae* (ESBL-E) carriage were identified from 300 adults recruited in the study, which gave a prevalence of 16.67% (50/300, 95% CI=12.43-20.91%). The mean age ±SD was 32.41±12.07 years; range, 18-75 years and 54.33% (163/300) were women. The results of unadjusted logistic regression model fitted to identify factors associated with ESBL-E carriage in community patients showed that there was no any degree of association between carriage of extended-spectrum β-lactamase producing *Enterobacteriaceae* in community patients with either their demographic or clinical characteristics.

Introduction

Extended-spectrum β-lactamase (ESBL) produced by many Gram-negative bacteria mediate resistance against penicillins, extended-spectrum cephalosporins and monobactams [1]. Infections caused by ESBL-producing pathogens has become a public health problem in different countries causing longer hospitalization, increased healthcare costs and higher morbidity and mortality as a result of the decreased therapeutic value of most common antibiotics used to manage patients [2–4].

While the acquisition of ESBL-producing pathogens was initially considered nosocomial [5] , the current trends in antimicrobial resistance continue to show increased evidence of higher rates of ESBL-producing *Enterobacteriaceae* (ESBL-E) carriage in the community settings [6–14].

Studies conducted to define the risk factors for acquiring infection caused by ESBL-producing bacteria in the community settings are very limited. Of the factors for the introduction of ESBL-E into the community that have been identified included 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* [15–17].

Transmission and spread of ESBL-producing *Enterobacteriaceae* strains in the community have also been suggested to occur through the food chain and companion animals [18–21]. A study conducted in Dutch patients, retail chicken meat and poultry revealed that human and poultry shared the same ESBL genes, plasmids and strains, this alone indicated that carriage of ESBL-E in food-producing animals, contamination of retail meat and environment can contribute to higher incidences of infections with ESBL-producing bacteria in humans [20]. However, indefinite carriage of ESBL-E overtime varies between persons and may increase the risk of community acquisition and transmission of ESBL pathogens. Furthermore, poor hygiene and weak implementation of antimicrobial policy perpetuate the spread of antibiotic resistance in the community [16].

Previous studies in Malawi have estimated the prevalence of invasive and carriage ESBL-producing *Enterobacteriaceae* isolated from blood cultures of hospitalized patients to range from 0.7% in 2005 to 90.5% in 2017 [22, 23]. Despite the increase of prevalence which was exclusively reported in hospital settings, no study has been conducted in Malawi and Blantyre in particular reporting predictive factors of ESBL-producing *Enterobacteriaceae* in either hospital or community settings. Investigating predictors of contracting strains of ESBL-producing *Enterobacteriaceae* in community settings is potentially important to understand causal mechanisms that can guide formulation and implementation of successful infection and antimicrobial use control strategies and empirical ESBL targeted antimicrobial therapy to both community and hospital patients. Therefore, this study examined factors associated with carriage of extended-spectrum β-lactamase producing *Enterobacteriaceae* in community patients in Blantyre, Malawi.

Material And Methods

Study design and setting

This was a cross-sectional study carried out between March and September 2020 to assess factors associated with extended-spectrum β-lactamase producing *Enterobacteriaceae* in randomly selected community patients attending outpatient health centres in Blantyre, Malawi. Three health centres were selected randomly. They included Limbe, Zingwangwa and Ndirande health centres.

Study population, Sample collection and laboratory procedures

The study participants comprised of 300 adult (≥18 years old) community patients. Participats present on the day of data collection were recruited randomly into the study regardless of their reason to seek health care. Social demographic characteristics and clinical data including age, sex, education, occupation, history of prior hospitalization, history of surgery and prior history of antibiotic use were collected using a standadard questionnaire. From each participant, either rectal swab or urine sample was collected for ESBL-E screening. Urine samples were collected exclusively from patients that had complained of UTI symptoms. Samples were taken using standard microbiological procedures and were immediately sent to the microbiology laboratory of the College of Medicine University of Malawi for processing.

Initial screening for potential ESBL-producing *Enterobacteriaceae* was performed by culture on chromogenic selective medium (CHROMagar^{TM} ESBL) supplemented with ESBL supplement containing a selective mixture of antibiotics enabling selective growth of ESBL-producing *Enterobacteriaceae* and inhibiting the growth of non-ESBL *Enterobacteriaceae* (CHROMagar^{TM}, Paris, France). The putative culture of ESBL producers was phenotypically confirmed using combination disk test method (CDT) by comparing the inhibition zone diameter around cefotaxime (CTX-30µg) and ceftazidime (CAZ-30µg) disks with and without clavulanic acid as previously described [24].

Biochemical identification of *Enterobacteriaceae*

Presumably, identification of common ESBL-producing *Enterobacteriaceae* isolates was first done based on bacterial colonial morphology and chromogenic characteristics on CHROMagar™ medium plates according to the manufactures' instructions. Subsequently, the identity of *Enterobacteriaceae* was confirmed using the commercially acquired biochemical substrate strips (Microbact™, Oxoid, GNB 12A) according to the manufacturer's instructions.

For quality control purposes, ESBL-producing *Klebsiella pneumonia* (ATCC 700603) and Non- ESBL producing *E. coli* (ATCC 25922) were used as positive and negative control respectively. **Statistical analysis**

The summary and descriptive statistics were generated as percentages, proportions, mean and standard deviation. Dichotomous variables were compared using Pearson's chi-square test or Fisher exact test as appropriate and continuous variables were compared using the Student's *t*-test. To identify the association between patients characteristics and carriage of ESBL-producing *Enterobacteriaceae*, the univariate logistic regression was used. A p-value ≤ 0.05 was considered statistically significant. Effect sizes of associations of patients characteristics and ESBL-E carriage were reported using Odd ratios (OR) and 95% confidence intervals (CI). During logistic regression analysis, 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. History of admission prior to data collection was omitted from the model because all individuals with confirmed ESBL-E phenotypes (dependent variable) had no history of admission in the past three months. All statistical analyses were performed with STATA version 12 (Stata Corp., College station, Texas, USA).

Results

Characteristics of study participants

A total of 50 community patients with ESBL-producing *Enterobacteriaceae* (ESBL-E) carriage were identified from 300 adults recruited into the study, which gave a prevalence of 16.67% (95% CI=12.43-20.91%). The average age ±standard deviation of participants was 32.41±12.07 years; range, 18-75 years and 54.33% (163/300) were women. Of the 50 patients with ESBL-E phenotype, the prevalence of ESBL-E was higher in males 56% (28/50), married or cohabiting 56% (28/50), unemployed 48% (24/50) and those with primary education 46% (23/50) (table 1).

The association between ESBL-E carriage in community patients and their social-demographic characteristics were statistically insignificant. Neither prior antibiotic use (OR= 0.87, 95%, CI: 0.41-1.84) nor the history of surgery three months before the study (OR=1.35, 95%, CI: 0.52-3.49) was associated with carriage of ESBL-producing *Enterobacteriaceae* in community patients.

Table 1: Independent non-predictors of ESBL-producing *Enterobacteriaceae* in community patients in Blantyre Malawi.

Factor	ESBL-positive (n=50)	ESBL-negative (n=250)	OR (95%, CI)	p-value †
Mean Age (mean±SD)	34.2±11.63	32.1±14.04	1.01(0.99-1.04)	0.25
Sex (Male) n(%)	28(56.00)	109 (43.60)	1.65(0.89-3.04)	0.11
Marital status n(%)				
Married or cohabiting	28 (56.00)	156 (62.40)	0.77(0.41-1.42)	0.39
Education level n(%)				
Primary	23 (46.00)	110 (44)	0.42(0.07-2.42)	0.33
Secondary	16 (32.00)	99 (39.60)	0.32(0.05-1.91)	0.21
Did no attend to any school	9 (18.00)	37(14.80)	0.49(0.08-3.08)	0.45
Occupation n(%)				
Employed	8(16.00)	70(28.00)	0.4(0.12-1.28)	0.12
Self-employment or business	12(24.00)	45(18.00)	0.92(0.31-2.82)	0.90
Unemployed	24(48.00)	114(45.60)	0.74(0.23-2.02)	0.55
Antibiotic use in the past 3 months n(%)				
Yes	10 (20.00)	56 (22.40)	0.87(0.41-1.84)	0.71
Surgery in previous 3 months n(%)				
Yes	6 (12.00)	23 (9.20)	1.35(0.52-3.49)	0.54

†Chi square test for dichotomous variables and Student's t test for continuous variables

Discussion

Our data showed 16% prevalence of ESBL-E in community patients in Blantyre and there were no significant factors associated with ESBL-E carriage. Previous studies have suggested that the prevalence

of ESBL-E in communities vary widely by geographic region and settings [25, 26]. Although hospitalized patients carrying hospital-acquired ESBL-producing bacteria over an extended period have been linked with the spread of ESBL-E to the community [27, 28], community emergence of extended-spectrum β-lactamase producing E*nterobacteriaceae* could arise from irrational antibiotic use by community patients [29].

In the current study, we found low prevalence of ESBL-E in community patients with no prior history of hospital admissions. This is an indication that patients from the community in Blantyre were likely to have been exposed to several courses of antibiotics due to irrational use as a result of weak restrictions and over the counter availability. Consequently, community patients could have acquired ESBL-E through selection from the existing gastrointestinal flora after antibiotics exposure [30]. Similar low prevalence of ESBL-E was reported in other studies [11, 14, 28, 31–33]. The probable explanation for the low prevalence of ESBL-E in community patients detected in this study could be lack of patients' prior history of hospitalization which have been reported as the main factor driving the spread of ESBL pathogens in the community.

While the current study highlights the lack of association between ESBL-E carriage in community patients and their clinical or social-demographic characteristics, several risk factors for community-acquired ESBL-E infections have been identified. These included the history of recurrent UTIs, urinary catheter placement, previous hospital admission, outpatient exposure to β-lactams (e.g. penicillins, cephalosporins) and quinolones, comorbidities, old age, male gender, and travel to areas with high rates of ESBLs infections [11, 25, 26, 34–39]. Similar to our findings, a study by Sanneh *et al.*, [40] did not find an association between demographic characteristics and ESBL-E Carriage in the community settings. Neither admission in the hospital, nor close contact with hospitalized individuals was significantly associated with the carriage of ESBL-E in other studies [41, 42]. We anticipate that these factors may have a causal relationship with ESBL-E carriage but may only lack statistical significance association because most of them have validity and biologic plausibility for a causal relationship with ESBL-E carriage as previously described [4, 26, 39, 43].

In previous studies, the male gender was reported as a risk factor for ESBL-E carriage [8, 44]. However, in this study, males had a higher proportion of ESBL-E carriage than women but male gender was not a statistically significant predictor of ESBL-E.

Conclusion

The current study provides evidence that ESBL-producing *Enterobacteriaceae* carriage is prevalent in the community in Blantyre. Nevertheless, factors responsible for this carriage remain unidentified. Even though further investigations including large case-control and molecular studies using one health approach are required to confirm community-based transmission of ESBLs and to determine the risk factors, reservoirs and vehicles for the dissemination of ESBL within the community in Blantyre Malawi;

the findings of the current study can answer the question on the importance of routine screening for ESBL producing pathogens to aid ESBL-E targeted antimicrobial therapy.

Limitations

This study was limited to phenotypic identification of ESBL-producing *Enterobacteriaceae.* However, the use of combined disk testing method (both cefotaxime and ceftazidime disks) to confirm ESBLs in this study was important because ESBLs could be missed if just a single disk was used. Low rate of EBLS-E carriage and type of study used could also limit the identification of the risk factors for ESBL-E carriage in the community because identification of risk factors for the carriage of ESBL-producing pathogens is most suitable with large infection rates and case-control study than a cross-sectional study used in the current study.

Declarations

Ethics approval and consent to participate

This study was approved by the College of Medicine Research Ethics Committee (COMREC) of the University of Malawi (Approval No. P.07/19/2720 of November 22, 2019). Blantyre district health authority granted permission to conduct research in health centres. Written informed consent was obtained from participants before enrolment into the study.

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. SFR, RSM, GKB and SA reviewed and contributed to content. All authors approved the final manuscript.

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