Lack of association between patients characteristics and carriage of extended-spectrum β-lactamase producing Enterobacteriaceae in community settings in Blantyre, Malawi

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Research note

Keywords: ESBL carriage, Enterobacteriaceae, Community, associated factors, Malawi

Posted Date: August 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-322264/v2

Abstract

Background: Infections caused by extended-spectrum β-lactamase (ESBL) producing bacteria are global health threat contributing to increased morbidity and mortality in resource-constrained countries. This cross sectional study examined factors associated with the carriage of ESBL-producing Enterobacteriaceae (ESBL-E) in community patients in Blantyre, Malawi.

Methods: We collected rectal swabs and urine samples from randomly recruited participants and screened for ESBL-E on CHROMagar^{TM} ESBL medium (CHROMagar, Paris, France). The ESBL-E isolates were identified using commercially acquired biochemical strips (Microbact™ GNB, Oxoid, UK) and production of ESBL was confirmed by the combination disk test using cefotaxime and ceftazidime disks with and without clavulanic acid. Antibiotic susceptibility test was done by the disc diffusion method and interpreted according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) 2019. Univariate logistic regression was used to evaluate association between ESBL-E carriage and the assosiated factors. To quantify relationships, 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. Results were expressed as odds ratio (OR) with a 95% confidence interval

Results

A total of 50 community patients with ESBL-E phenotypes were identified from 300 adults recruited in the study, which gave a ESBL-E prevalence of 16.67% (50/300, 95% CI=12.43-20.91%). The mean age ±SD of participants was 32.41±12.07 years; range, 18-75 years and 54.33% (163/300) were women. on unadjusted logistic regression, no association between carriage of ESBL-E and community patient characteristics was observed.

Conclussions

The carriage of ESBL-E is prevalent in the community in Blantyre-Malawi. Nevertheless, factors associated with this carriage remain unidentified. 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 factors, reservoirs and vehicles associated with the dissemination of ESBL within the community in Blantyre, Malawi.

Introduction

Extended-spectrum β-lactamase 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-E was initially considered nosocomial [5] , the current trends in antimicrobial resistance continue to show increased evidence of higher rates of ESBL-E carriage in the community settings [6–14].

Studies conducted to define the risk factors for acquiring infection caused by ESBL-E 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-E, previous hospitalization, antibiotic treatments, old age, comorbidities like diabetes and previous infection by members of *Enterobacteriaceae* [15–17].

Transmission and spread of ESBL-E 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 stains genes and plasmids, 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]. Even though carriage of ESBL-E differs between persons, indefinite carriage overtime 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-E isolated from blood cultures of hospitalized patients to range from 0.7% to 90.5% in 2005 and 2017 respectively [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-E in either hospital or community settings. Investigating predictors of contracting strains of ESBL-E 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 ESBL-E in community patients in Blantyre, Malawi.

Materials And Methods

Study design and setting

This was a cross-sectional study carried out between March and September 2020 to assess for the factors associated with ESBL-E carriage in randomly selected community patients attending outpatient health centres in Blantyre, Malawi. The three health centres that were selected randomly included Limbe, Zingwangwa and Ndirande health centres.

Study population and Sample collection

The study participants comprised of 300 adult (≥18 years old) outpatients. 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 purposely 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 at Kamuzu university of Heaalth Sciences (KUHeS) for laboratory procedures.

ESBL-E screening

Initial screening for potential ESBL-E was performed by culture on a chromogenic selective medium (CHROMagar^{TM} ESBL) supplemented with ESBL supplement containing a selective mixture of antibiotics enabling selective growth of ESBL-E and inhibiting the growth of non-ESBL-E (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-E isolates was done based on bacterial colonial morphology and chromogenic characteristics on CHROMagar^{TM} 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-E, 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).

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.

Results

Characteristics of study participants

A total of 50 community patients with 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; the age range was between 18 and 75 years old. Majority of participants were women 54.33% (163/300). The prevalence of ESBL-E was higher in male (9.33%) similar to married or cohabiting participants, 8% for unemployed and 7.6% for those with primary education (table 1).

Table 1: Characteristics of the study participants

Factors	Frequency n(%)	ESBL phenotype	
		Positive n(%)	Negative n(%)
Age			
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			
Male	137(45.67%)	28(9.33%)	109 (36.33%)
Female	163(54.33%)	22 (7.34%)	141(47.00%)
Marital status			
Single	116 (38.67%)	22 (7.34%)	94 (31.33%)
Married or cohabiting	184 (61.33%)	28 (9.33%)	156 (52.00%)
Education			
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			
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			
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 months			

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 months			
Yes	7 (2.33%)	0(0.00%)	7(2.33%)
No	293 (97.67%)	50 (16.67%)	243(81.00)
Outpatient health centre			
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)

Association of participant's characteristics and risk factors for ESLB-E carriage

The analysis of the risk factors for ESBL-E carriage and community patient characteristics showed statistically insignificant association. 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-E in community patients.

Table 2: Independent non-predictors of ESBL-producing *Enterobacteriaceae* in community patients in Blantyre, Malawi.

Factor	OR (95%, CI)	p-value †
Age (mean±SD)	1.01(0.99-1.04)	0.25
Sex (Male) n(%)	1.65(0.89-3.04)	0.11
Marital status n(%)		
Married or cohabiting	0.77(0.41-1.42)	0.39
Education level n(%)		
Primary	0.42(0.07-2.42)	0.33
Secondary	0.32(0.05-1.91)	0.21
Did no attend to any school	0.49(0.08-3.08)	0.45
Occupation n(%)		
Employed	0.4(0.12-1.28)	0.12
Self-employment or business	0.92(0.31-2.82)	0.90
Unemployed	0.74(0.23-2.02)	0.55
Antibiotic use in the past 3 months n(%)		
Yes	0.87(0.41-1.84)	0.71
Surgery in previous 3 months n(%)		
Yes	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], in the current study, community emergence of ESBL-E could arise from irrational antibiotic use by community patients [29].

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 ESBL 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 studies by Kurz *et al* and Briongos-Figuero *et al* respctively [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 associated with 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 associated 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.

Declarations

Acknowledgements

This research was supported by the Africa Centre of Excellence in Public Health and Herbal Medicine as part of the first author's Ph.D fellowship. The funders had no role in the design of the study, data collection, analysis and interpretation or writing the manuscript. A preliminary verion of this manuscript has been presented as preprint in Research square and can be accessed through the following link, https://www.researchsquare.com/article/rs-322264/v1

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.

Competing interests

The authors declare no competing interests

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