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Fecal carriage rates of extended-spectrum β -lactamase-producing *Escherichia coli* of inpatients and outpatients attending Yobe State Teaching Hospital, Damaturu, Nigeria

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ABSTRACT

Background: Extended-spectrum β -lactamases-producing *Escherichia coli* has been on rise and its infection is becoming difficult to treat. These resistant bacteria can colonize human gastrointestinal tract and can easily be spread in population. The aim of this study was to assess the fecal carriage rates of extended spectrum beta lactamase-producing *E. coli* (ESBL) among hospitalized and out-patients. **Methods:** A total of 200 volunteers participated in the study. A stool sample was collected from each participant and subjected to standard microbiological methods for the isolation of *E. coli*. Furthermore, the isolates were subjected to phenotypic detection of ESBL using double-disk synergy test. The confirmatory test was performed with CAZ (30 μ g), CTX (30 μ g) and CRO (30 μ g) around Augmentin disc (AMC 30 μ g) and clear zone of inhibitions towards the AMC (30 μ g) considered as positive results for production of ESBL. Basic demographic information were recorded. **Results:** From the 200 fecal samples collected, all tested positive for *E. coli*. 108 females participated which represents (54%) while 92 males participated which represents (46%) and their age group ranges between 21years to 40years (n =94/200; 47%). Among the participants, 113/200 (56.5%) were out-patients while 87/200 representing 43.5% were hospitalized patients. In this study, 116/200 (58%) of the 200 volunteers were found to harbored *E. coli* producing ESBL. In this study, age, gender, and hospitalization status play role in fecal carriage rates of ESBL-producing *E. coli*. **Conclusion:** The results of this study reveal high fecal colonization of ESBL-producing *E. coli*. Therefore, there is need for prudent use of antibiotics among hospitalized and out-patients.

Introduction

Escherichia coli are Gram-negative bacteria, member of *Enterobacteriaceae*. They inhabit gastrointestinal tract and mainly considered

harmless but are also known to cause number of hospitals associated infections [1]. Of interest, is the global rise in antibiotic resistance in *E. coli* making

a leading pathogen causing deaths due to infection associated with antibiotic resistance [2].

Extended-spectrum β -lactamases (ESBLs) are plasmid-mediated, making transfer of resistance among Gram-negative more common [3]. Recently, increase rate of infections with *Enterobacteriaceae*-producing ESBL, especially *E. coli* has been noted across the world [3], in Europe and North America showed trend of 10 and 15% increase respectively, while in Southeast and East Asia, the rates of 20-40% were reported [4]. Studies from parts Africa exhibit high rates of ESBL-*E. coli* in both hospital and community setting despite lack of effective surveillance systems [5]. In Nigeria, a West African country reported alarming prevalence of ESBLs-*E* [6]. Many studies reported the rates of ESBLs producing *Enterobacteriaceae* in Nigeria, and of particular concern is the alarming rates among gram-negative bacteria [7-9]. Despite the considerate reports of levels of ESBLs in Nigeria, only 11.6% is from this study area [9]. Thus, this study focused on the fecal carriage rates of ESBL-producing *Enterobacteriaceae* in clinical settings of Damaturu, Yobe State.

Materials and Methods

Study population

This study was conducted between May to September 2021, subjects were recruited from Yobe State University Teaching Hospital, Damaturu, Yobe State, Nigeria. The volunteers were both hospitalized and out-patients and the study was duly informed to the volunteers. A total of 200 participates were involved for fecal samples collections.

Ethical approval

Ethical approval for the study was obtained from Research committee of Yobe State University Teaching Hospital. Written informed consent was collected from each participants.

Sample and data collection

From each volunteer, 2g of stool sample was collected in a transparent container and although the procedure was explained to them. During the sample collection, a questionnaire was applied to each participant to retrieved basic demographic data such as age, gender and hospitalization status.

Sample collection, preparation and identification

The stool samples were aseptically collected using sterile universal containers (200ml) and assigned with study number. All the clinical samples

collected were streak separately onto MacConkey agar plates under aseptic techniques and incubated at 37 °C for 24 hours. The resulting colonies were further sub-cultured for confirmatory onto Eosin Methylene Blue agar and incubated at 37 °C for 24 hours. Subsequently, Gram staining and biochemical techniques were employed to identify specific colonies.

Preliminary identification

Preliminary identification included Gram staining and biochemical tests which include; citrate test, methyl red test, triple sugar iron test and indole test.

Primary test for production of extended spectrum β -lactamase

The antibiotic susceptibility testing was observed in three types of antimicrobial agents (3rd generation cephalosporins); ceftazidime (CAZ 30 μ g), cefotaxime (CTX 30 μ g) and ceftriaxone (CRO 30 μ g). If inhibition zone for bacterial isolates were: <27 mm for CAZ, <22 mm for CTX and <25 mm for CRO, this result considered as positive result for production of extended spectrum beta lactamase (10CLSI, 2016).

Confirmatory test for extended spectrum β -lactamase

The confirmatory test was performed with Augmentin disc (AMC 30 μ g) placed at center of Mueller Hinton/ Nutrients agar plate containing the streaked colonies of the positive isolate. Three discs include; CAZ (30 μ g), CTX (30 μ g) and CRO (30 μ g) were placed at sides of Augmentin (AMC 30 μ g) disc with distance of fifteen (15) mm from center and the plate was incubated overnight at 37°C for 24 hours. The clear zone of inhibitions towards the AMC (30 μ g) considered as positive results for production of ESBL [10].

Results

A total of 200 *Escherichia coli* isolates were turned positive from two all stool samples. Of the total 200 volunteers, 92 (46%) were male and 108 (54%) were female. The age distributions of the volunteers were as follows; age 1-20 (n =37/200; 18.5%), 21-40 (n =94/200; 47%), 41-60 (n =46/200; 23%), 61-80 (n =22/200; 11%) and age group 81-100 (n =1/200; 0.5%). Among the participants, 113/200 (56. 5%) were out-patients while 87/200 representing 43.5% were hospitalized patients. In this study, 116 (58%) of the 200 volunteers were found to harbored *E. coli* producing ESBL. On gender distribution of fecal carriage of ESBL- *E. coli*, 59/116 (50.9%) were males while 57/116

(49.1%) were females. Furthermore, age group 21-40 had the highest ESBL- *E. coli* 58/116 (50.0%), followed by age group 41-60 23/116 (19.8%), age group 1-20, (17.1%), age group 61-80 (12.1%) while age group 81-100 had not been colonized by ESBL-*E. coli*. Of 116 ESBL-producing *E. coli* isolates, 52/116 (44.8%) were found to be among hospitalized patients while 64/116 (55.2%) were accounted for out-patients.

Discussion

Fecal carriage of ESBL-producing *E. coli* is a major threat to global public health and increase antibiotic resistance burden. This is of concern because colonization of resistant *E. coli* from gut can be disseminated not only in clinical setting but to environment and subsequently to community and keep spreading. Therefore, the aim of the present study was phenotypic and genotypic characterization of fecal carriage ESBL-producing *E. coli* obtained from hospitalized patients and out-patients. It is important to assess the fecal carriage of ESBL-producing *E. coli* among patients which will add to the global surveillance of antibiotic resistant bacteria of public health importance.

In the current study, of the 200 stool samples collected, all returned positive for *E. coli*. This is not surprising as high fecal carriage of members of *Enterobacteriaceae* is documented worldwide, also being member of gut microbiome [11].

Fecal carriage of ESBL-producing *Enterobacteriaceae* has been reported globally at varying degree, with Asia having highest prevalence while Europe and North America accounted for lowest prevalence [1,11]. The exact level of prevalence of ESBL-producing *Enterobacteriaceae* in sub-Saharan Africa is difficult to ascertain due to lack of adequate data and poor resources, however, there is steady raise in fecal carriage of ESBL-Producing *E. coli* [5,11]. A study from Nigeria reported high prevalence of ESBL-producing *E. coli* of 82.3% [9], this finding is not surprising as the current study reported 58% of all the fecal samples to collected harbored ESBL-producing *E. coli*. On the hand, the prevalence of ESBL-E carriage in Europe is reported to be lower [12]. By the current results, ESBL-producing is public health threat and will continue to circulate in the study area.

Regarding gender distribution of ESBL among *E. coli* derived from stool samples, the current study showed higher levels in females 108 (54%) than in males 92 (46%). This is consistent with other reports [13-15] which showed higher rate of ESBL-producing *E. coli* in females than males.

The rate of ESBL-producing *E. coli* in the current study was higher among age group 20-40 (47%) years and least among elderly 80-100 years old (0.5%). Contrary to the current study, **Letara et al.** [16] detected higher prevalence (48%) among children aged than 15 years. This variability could be accounted on basis of the population of target.

As for the hospitalization status, the current findings detected 64/116 (55.2%) among the out-patients which is higher than hospitalized 52/116 (44.8%). This could be due to unregulated use of antibiotics which might be higher among out-patients. Also, asymptomatic carriage among discharged patients as reported elsewhere [17]. This could point out to the fact that out-patients are coming from community that circulate the ESBL and its reservoir.

Conclusion

The results in this study revealed high prevalence of ESBL-producing *E. coli* which calls for concern. Therefore, it is important that all stakeholders should deploy ways of controlling continued spread of antibiotic resistant bacteria and resistant genes.

Conflict of interest

We declare that we have no conflict of interest.

Financial disclosures

Nothing to declare

Authorship

Each author listed in the manuscript had contributed and approved the submission of this version of the manuscript and takes full responsibility for it.

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