Acta Scientific Microbiology (ISSN: 2581-3226)

Volume 1 Issue 8 August 2018

Research Article

Extended Spectrum B-Lactamase (ESBL) Expression in *Escherichia coli* Isolates from Anal Swabs of Donkeys in a Local Donkey Abattoir in Abakaliki, Nigeria

Ovia Kenneth[1], Beatrice Ugbo[1], Iroha Ifeanyichukwu[2] and Chika Ejikeugwu[2]*

[1]*Department of Biological Science (Microbiology), Evangel University Akaeze, Abakaliki, Ebonyi State, Nigeria*

[2]*Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria*

***Corresponding Author:** Chika Ejikeugwu, Department of Applied Microbiology, Faculty of Sciences, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.

Received: April 20, 2018; **Published:** July 02, 2018

Abstract

Extended spectrum beta-lactamases (ESBLs) are enzymes that give some Gram-negative bacteria such as Escherichia coli and Klebsiella species the exceptional ability to resist the antimicrobial action of third-generation cephalosporins including cefotaxime, ceftazidime and ceftriaxone. This study detected the presence of ESBL-producing E. coli isolates from anal swabs of donkeys in a local donkey abattoir. A total of thirty (30) anal swab samples were collected from the anal region of the donkeys; and these were bacteriologically analyzed for the isolation of E. coli. All samples were cultured on MacConkey agar plates and eosin methylene blue agar plates for the isolation of E. coli isolates after prior cultivation in nutrient broth tubes. The E. coli isolates was identified using conventional microbiology techniques. ESBL production was detected phenotypically using the double disk synergy test (DDST) technique, and antimicrobial susceptibility testing was carried out using the Kirby-Bauer disk diffusion technique. A total of 25 E. coli isolates was isolated from the 30 anal swab samples. The E. coli isolates showed high resistance rates to the antibiotics especially to the 3rd-generation cephalosporins, and the monobactam, aztreonam. The result of the antimicrobial susceptibility testing showed that most of the E. coli isolates where found to resist the antibiotic action of some of the tested antimicrobial agents particularly cefoxitin (32 %), ceftriaxone (60 %), cefotaxime (100 %), cefuroxime sodium (100 %), aztreonam (100 %) and cefepime (66.7 %). Out of the 25 E. coli isolates phenotypically screened for ESBL production, only 10 E. coli isolates were suspected to produce ESBL by the screening test using cefotaxime, and cefuroxime, ceftriaxone as the screening antimicrobial agents. However, ESBL production was phenotypically confirmed in only two (2) isolates of E. coli (representing 8 % of the total isolates) using the DDST technique. In conclusion, our study presumptively revealed that the E. coli isolates from this particular donkey abattoir are multidrug resistant in nature; and they also express ESBL phenotypically. Adequate measures are required for the handling of animals in order to avoid horizontal transmission of the ESBL-producing bacteria to humans. However, the prohibition of the use of antibiotics in animal husbandry as well as proper hygienic practices are recommended as measures to keeping antibiotic resistant bacteria at bay.

Keywords: ESBLs; Gram Negative Bacteria; Escherichia coli; Antimicrobial Susceptibility Testing; Nigeria

Introduction

Antimicrobial resistance is a global health phenomenon that impacts negatively on our ability to effectively treat and manage infectious diseases especially those caused by pathogenic bacteria that produce extended spectrum β-lactamases (ESBLs) [1-4]. This changing evolutionary mechanism of bacteria to evolve resistance to antimicrobial agents also affects food security, healthcare and the economic development of any nation because it limits the number of choices for antimicrobial therapy and worsens the health conditions of patients. The prevailing problem of antimicrobial resistance knows no border of any nation and can easily spread from one region to another if left undetected and uncontrolled. Gram negative bacteria from abattoir including pathogenic Escherichia coli represent one of the most relevant reservoirs of antibiotic resistance genes in the environment; and they can easily transfer their resistance genes to susceptible bacteria in the environment through genetic transfer mechanisms. The rising rates of antimicrobial resistance across the globe calls for a concerted effort at both the national, regional and global levels to halt its effect and begin to reverse the harm that they cause on some available anti-

biotics in order to secure the therapeutic efficacy of these agents [1,2,5-8]. ESBLs are enzymes that hydrolyze extended - spectrum cephalosporins including cefotaxime, ceftriaxone and ceftazidime with an oxyimino side chain; but they are inhibited by clavulanic acid [2,3,9-11]. They occur mostly in Gram negative bacteria especially members of the Enterobacteriaceae family such as Escherichia coli and Klebsiella species; and ESBL-producing bacteria are associated with increased morbidity [1,6]. Though chromosomally mediated, ESBL production can also be plasmid-mediated; and ESBL-producing bacteria was first isolated in Germany in 1983 [12]. However, they have since then spread worldwide – limiting the efficacy of most antibiotics used for treating bacterial related infections [1,2,6,7,13]. Organisms producing ESBLs are now found in both the community and hospital environments; and the irrational use of antibiotics in animal husbandry and in other agricultural purposes could provide room for ESBL-producing bacteria to emerge and spread via selective pressure of antimicrobial usage. The introduction of the 3rd-generation cephalosporins into clinical practice in the early 1980s was heralded as a major breakthrough in the fight against β-lactamase-mediated bacterial resistance, but this important discovery in clinical medicine was not long lasting due to the emergence of drug resistant bacteria pathogens that now defy the antimicrobial efficacy of these agents [6,12-15]. Nevertheless, the emergence of ESBLs has led to the demise in the antimicrobial efficacy of some antibiotics, and this has put to threat the efficacy of other non β-lactam agents in treating bacterial related infections since ESBL-producing bacteria may be multidrug resistant in nature. Their incidence has been steadily increasing over the years resulting in the limitation of therapeutic options [1,2,13-15]. More worrisome is the occurrence of these organisms in non-hospital environments, which may be attributed to the irrational use of antibiotics for non-medical purposes. Drug resistant bacteria continue to be a major problem for health personnel's around the world due to the challenges experienced in selecting appropriate therapy for treatment since resistant bacteria are ever evolving in their antimicrobial resistance mechanisms. It is therefore important for microbiology laboratories in Nigeria to detect these organisms in their routine susceptibility studies using prompt and accurate detection techniques that will only select for drug resistant bacteria pathogens from clinically important samples. In this study, we reported presumptively, the occurrence of ESBL-producing *E. coli* from anal swabs of donkeys in Abakaliki, Nigeria using phenotypic detection protocol.

Materials and Methods

Samples

The samples used for this study was anal swabs from the rectal region of donkeys due for slaughter in a local abattoir in Abakaliki, Nigeria. A total of 30 anal swab samples were collected from the donkeys; and they were transported to the microbiology laboratory of Evangel University Akaeze, Ebonyi State, Nigeria within 1 hour for bacteriological analysis.

Culture and identification of bacteria

The anal swab samples were each dipped into test tubes containing 5 ml of freshly prepared nutrient broth (Oxoid, UK) and the tubes were loosely covered with cotton wool and incubated at 35oC for 18 - 24 hours. Bacterial growth was observed by the presence or absence of turbidity in the nutrient broth tubes; and a loopful of the turbid solution in the nutrient broth tubes was aseptically subcultured onto freshly prepared MacConkey agar and eosin methylene blue (EMB) agar plates (Oxoid UK) and incubated at 35°C for 18 - 24 hours. *E. coli* was identified using standard microbiology identification techniques including colonial morphology, Gram staining and biochemical testing [16].

Antibiotic susceptibility testing

This was aseptically carried out on Muller-Hinton agar (Oxoid, UK) plates using disk diffusion method, and in conformity to the recommended standard of Clinical and Laboratory Standard Institute [18]. The antibiotic disks used were: imipenem (10 µg), ceftriaxone (30 µg), cefotaxime (30 µg), cefoxitin (30 µg), amoxicillin-clavulanic acid (20/10 µg), cefuroxime sodium (30 µg), aztreonam (30 µg) and cefepime (10 µg) (Oxoid, UK). All susceptibility plates were incubated at 35°C for 18-24 hours. Percentage susceptibility and resistance was interpreted from the inhibition zone diameters (IZDs) produced by the antibiotic disks against the test isolates [17,18].

Detection of ESBL positive *E. coli*

ESBL production was phenotypically confirmed in only the *E. coli* isolates that showed reduced susceptibility to the 3rd-generation cephalosporins (such as cefotaxime and ceftriaxone) using the double disk synergy test (DDST) technique [3,18]. Standardized inoculum of *E. coli* (adjusted to 0.5 McFarland turbidity standards) was aseptically swabbed on the MH agar plates; and amoxycillin-clavulanic acid disc (20/10 µg) was placed at the centre of the plate while cefotaxime (30 µg) and ceftazidime (30 µg) discs were each adjacently placed at a distance of 15 mm away from the amoxycillin-clavulanic acid disc. The plates were incubated at 37°C for 18 - 24 hrs; and ESBL production was phenotypically inferred by the determination of a difference of ≥ 5 mm increase in the inhibition zone diameter when the cephalosporins was used alone and in combination with amoxicillin-clavulanic acid [2,3].

Results

Table 1 shows the result of the biochemical tests and morphological identification of the isolated Escherichia coli isolates recovered from the anal swab samples of donkeys in this study. A total of 25 E. coli isolates was bacteriologically recovered from the anal swab samples analyzed. E. coli is an enteric organism that is ubiquitous in the gastrointestinal tract of humans and animals; and pathogenic strains of the organism are usually responsible for some community acquired infections such as urinary tract infections (UTIs). The result of the antimicrobial susceptibility testing of the isolated E. coli isolates is shown in table 2. The isolated E. coli isolates showed varying levels of resistance and susceptibility to the tested antibiotics; but they were found to be highly resistant to aztreonam, cefepime, cefuroxime, cefotaxime and ceftriaxone (Table 2).

Sample Source	Organism (n = 30)	n (%)	Morphological appearance	Gram reaction	Indole test
Anal swabs of donkey	*Escherichia coli*	25 (52.5)	Pink, lactose fermenting colonies on MAC; and metallic sheen colonies on EMB	Negative	Positive

Table 1: Characterisation of E. coil isolates.

Key: MAC: MacConkey Agar, EMB: Eosin Methylene blue; n: Number of Isolates; %=Percentage.

Antibiotics n (%)	Susceptible n (%)	Resistant
IMP (10 µg)	25 (100)	0 (0)
AMC (20/10 µg)	20 (80)	5 (20)
FOX (30 µg)	7 (68)	8 (32)
CRO (30 µg)	10 (40)	15 (60)
CTX (30 µg)	0 (0)	25 (100)
CXM (30 µg)	0 (0)	25 (100)
ATM (30 µg)	0 (0)	25 (100)
FEP (10 µg)	0 (0)	25 (100)

Table 2: Results of antimicrobial susceptibility testing.

KEY: IMP-Imipenem; AMC: Amoxicillin-Clavulanic Acid; CRO: Ceftriaxone; CTX: Cefotaxime; FOX: Cefoxitin; CXM: Cefuroxime Sodium; ATM: Aztreonam; FEP: Cefepime.

Organism	No of isolates tested	ESBL positive n (%)	ESBL negative n (%)
E. coli	25	2(8)	8(92)

Table 3: DDST detection of ESBL in 25 isolates of *E. coli.*

Discussion

Escherichia coli is a well-known member of the *Enterobacteriaceae* family of bacteria; and even though many strains of Escherichia are commensal in nature; a handful of *E. coli* strains can be pathogenic in nature and even carry and transmit antibiotic resistance genes in their environment. More so, *E. coli* is usually implicated as a major causative agent in some water borne and/or food borne infections, in which most of the outbreaks has been attributed to poor food management and poor hygienic practices during food processing. As an enteric organism, *E. coli* is a common indicator in faecal contamination of water bodies; and strains of *E. coli* harbouring antibiotic resistance genes such as those responsible for the production of extended spectrum β-lactamases (ESBLs) could worsen the clinical condition of people infected by the organism due to their antibiotic resistant nature. In this study, the frequency of ESBL-positive *E. coli* isolates recovered from the anal swab samples of donkeys due for slaughter in a local abattoir in Abakaliki, Nigeria was bacteriologically and phenotypically investigated. Out of the 30 anal swab samples bacteriologically analyzed in this study, a total of 25 isolates of *E. coli* was recovered; and these isolates also produced varying rates of susceptibility and resistance to the antimicrobial agents tested against them. *E. coli* have been reported as an important pathogen in the community and is responsible for a handful of community acquired infections [2,19,20]. Proper boiling and cooking of donkey meat products is important to avoid the contamination or contraction of infection through them. The *E. coli* isolates showed very high levels of resistance to the tested antibiotics particularly to cefepime, aztreonam, cefuroxime and cefotaxime – to which they were completely resistant to and had a resistance rate of 100 %. However, none of the *E. coli* isolates showed reduced susceptibility to imipenem, a carbapenem which is used to treat infections caused by ESBL-producing bacteria [1,8,11]. Imipenem followed by amoxicillin-clavulanic acid and cefoxitin were the best performing antibiotics in terms of their antimicrobial activity against the *E. coli* isolates used in this study. Out of the 25 E. coli isolates bacteriologically recovered from the anal swab samples of the donkeys, ESBL production was phenotypically detected in only 2 (8 %) isolates. The other 23 isolates of *E. coli* were not phenotypically confirmed ESBL producers by the DDST technique used in our study. The prevalence of ESBL-producing bacteria in non-hospital environment such as abattoirs

and poultry farms have been previously reported [3,4,9,13,19-21]. This study is one among the many reports of the prevalence of ESBL producing bacteria in the non-hospital environment in Nigeria; and this could be attributable to the irrational usage of antimicrobial agents in animal production and in veterinary practices, which has allowed resistant strains of bacteria including those that produce ESBLs to emerge and spread. This scenario is of public health importance because ESBL detection and the detection of other forms of multidrug resistant bacteria is not yet a routine laboratory practice in our local hospitals; and the country still has no national plan to contain the irrational use of antibiotics especially for non-human purposes. Our study is only but a preliminary study and may not be a reflection of what is actually happening in the study area. However, there is need to be on the lookout for drug resistant bacteria in this part of the world and in Nigeria as a whole – owing to the fact that antibiotics are obtained over-the-count (OTC) even without a doctor's prescription; and the usage of antibiotics for animal husbandry, poultry production and in other agricultural and veterinary practices allow resistant organisms including those that produce ESBLs to emerge and spread.

Conclusion

In conclusion, our study presumptively revealed that the *E. coli* isolates from this particular donkey abattoir are multidrug resistant in nature; and they also express ESBL phenotypically. Adequate measures are required for the handling of animals in order to avoid horizontal transmission of the ESBL-producing bacteria to humans. However, the prohibition of the use of antibiotics in animal husbandry as well as proper hygienic practices are recommended as measures to keeping antibiotic resistant bacteria at bay.

Bibliography

1. Jacoby GA., *et al*. "Mechanisms of Disease: The New β – Lactamases". *The New England Journal of Medicine* 352.4 (2005): 380-391.

2. Ejikeugwu C., *et al*. "Detection of metallo-β-lactamase (MBL) among carbapenem-resistant Gram-negative bacteria from rectal swabs of cow and cloacae swabs of poultry birds". *Annals of Medical and Health Science Research* 7 (2017): 51-56.

3. Ejikeugwu C., *et al*. "Phenotypic Detection of Klebsiella pneumoniae Strains - Producing Extended Spectrum β-Lactamase (ESBL) Enzymes". *Scholars Academic Journal of Biosciences* 1 (2013a): 20-23.

4. Ejikeugwu C., *et al*. "Detection and antimicrobial susceptibility of some Gram-negative bacteria producing carbapenemases and extended spectrum beta lactamases". *International Journal of Microbiology and Immunology Research* 2.6 (2013b): 064-069.

5. Ejikeugwu C., *et al*. "First Detection of FOX-1 AmpC β-lactamase gene expression among Escherichia coli isolated from abattoir samples in Abakaliki, Nigeria". *Oman Medical Journal* 33.3 (2018): 134-140.

6. Chroma M., *et al*. "Genetic Methods for Detection of Antibiotic Resistance: Focus on Extended – Spectrum β – lactamases". Biomedical Papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia 154.4 (2010): 289-296.

7. Chouchani C., *et al*. "VIM and IMP metallo-β-lactamases and other extended-spectrum β-lactamases in Escherichia coli and Klebsiella pneumoniae from environmental samples in a Tunisian hospital". *APMIS* 119.10 (2011): 725-732.

8. Peter CE., *et al*. "Extended-spectrum β-lactamase-producing Escherichia coli isolates from suspected community acquired urinary tract infections". *European Journal of Scientific Research* 84 (2012): 565-571.

9. King-Ting L. "Characterization of Multidrug Resistant ESBL – producing Escherichia coli isolates from Hospitals in Malaysia". *Journal of Biomedicine and Biotechnology* (2009): 1-10.

10. Alistair S. "Extended spectrum β - lactamases: Are we prepared to face the threat". *SCIEH Weekly Report* 38 (2004): 1.

11. Bradford PA. "Extended spectrum β – lactamases in the 21st century: characterization, epidemiology, and detection of this important resistant threat". *Clinical Microbiology Review* 14.4 (2001): 933-951.

12. Knothe H., *et al*. "Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of Klebsiella pneumonia and Serratia marcescens". *Infection* 11.6 (1983): 315-317.

13. Zhang L. "Acquired Antibiotic Resistance: Are We Born with It?". *Applied and microbiology AEM* 77.20 (2011): 7134-7141.

14. Zhao J., *et al*. "Antimicrobial resistance in enterococci isolates from pet dogs in Xi'an, China". *Pakistan Veterinary Journal* 32 (2012): 462-464.

15. White DG., *et al*. "Emergence and Transfer of Antibacterial Resistance". *Journal of Dairy Science* 84 (2001): E151-E155.

16. Cheesbrough M. "District Laboratory Practice in Tropical Countries". 2nd edition Cambridge University Press, UK (2006): 178-187.

17. Ejikeugwu., *et al*. "Evaluation of antibacterial activity of the leave extracts of Buchholzia coriacea". *Asian Journal of Pharmaceutical and Biological Research* 2 (2012): 204-208.

18. Clinical Laboratory Standard Institute, CLSI. "Performance standards for antimicrobial disk susceptibility test. Fifteenth informational supplement, CLSI document M100-S15. CLSI, Wayne, PA. USA (2011).

19. Ramos S., *et al*. "Clonal diversity of ESBL producing Escherichia coli in pigs at slaughter level in Portugal". *Foodborne Pathogens and Disease* 10.1 (2013): 74-79.

20. Wallensten A., *et al*. "Extended spectrum beta-lactamases detected in Escherichia coli from gulls in Stockholm, Sweden". Infection Ecology and Epidemiology 1 (2011): 7030.

21. Ewers C., *et al*. "Extended-spectrum beta-lactamase-producing and Amp C-producing Escherichia coli from livestock and companion animals, and their putative impact on public health: a global perspective". *Clinical Microbiology and Infection* 18.7 (2012): 646-655.

Volume 1 Issue 8 August 2018

© All rights are reserved by Chika Ejikeugwu*., et al.*